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## Mouse kidney development

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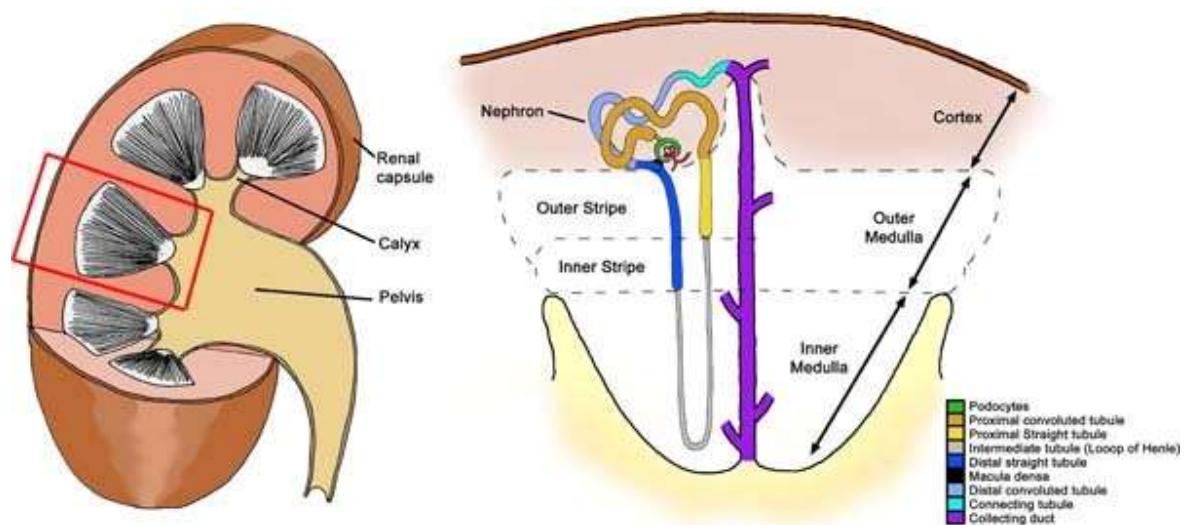
### Introduction

The kidneys play a critical homeostatic role in the regulation of body fluid composition and excretion of waste products. Here I review our understanding of how the three different vertebrate kidney types (pronephros, mesonephros, and metanephros) arise during mouse development with a more comprehensive focus on the molecular regulation of metanephros formation. A detailed understanding of the genetic hierarchies governing renal development will provide insights into the pathogenesis of kidney disorders, advance efforts to direct pluripotent stem cells into therapeutically useful renal lineages *in vitro*, and further our understanding of renal regenerative pathways that occur *in vivo*.

### Overview of kidney structure and embryonic development

The kidneys are bilateral organs that regulate the composition and volume of the body fluids, and eliminate metabolic waste products. The filtering unit of the kidney is the nephron, which has a characteristic segmental organization. The glomerulus at the proximal end of the nephron filters the blood and passes the filtrate to a tubular epithelium that modifies it before delivering the urine to the collecting duct for disposal (see Figure 1). Mammalian renal development differs from that of most other organs in that it proceeds through a series of three successive phases, each marked by the formation of a more complex pair of kidneys. These kidneys, which are called the pronephros, mesonephros, and metanephros, develop in a cranial (anterior)-to-caudal (posterior) progression from stripes of mesodermal cells, called the intermediate mesoderm, that extend from the heart region to the tailbud of the embryo (see Figure 2). In amniotes (birds, mammals, and reptiles) the pronephros and the mesonephros are generally transient embryonic kidneys that subsequently degenerate and have little or no functionality. The metanephros, which persists as the definitive adult kidney, is characterized by an extensively branched collecting duct system and

a large number of nephrons (~11,000 in the mouse and 300,000–1 million in humans (Nyengaard and Bendtsen, 1992; Yuan et al., 2002). Structurally, the mammalian metanephros comprises an inner medullary region (containing collecting ducts and long loops of Henle—the nephron segment involved in urine concentration), an outer medullary region (containing short Loops of Henle and collecting ducts), and a cortical region (containing all other domains of the nephron; Figure 1). This gross structure is important for the functionality of the metanephric kidney as it establishes an osmotic gradient between the cortex and medulla that drives the extraction of water from the urine (Fenton and Knepper, 2007).

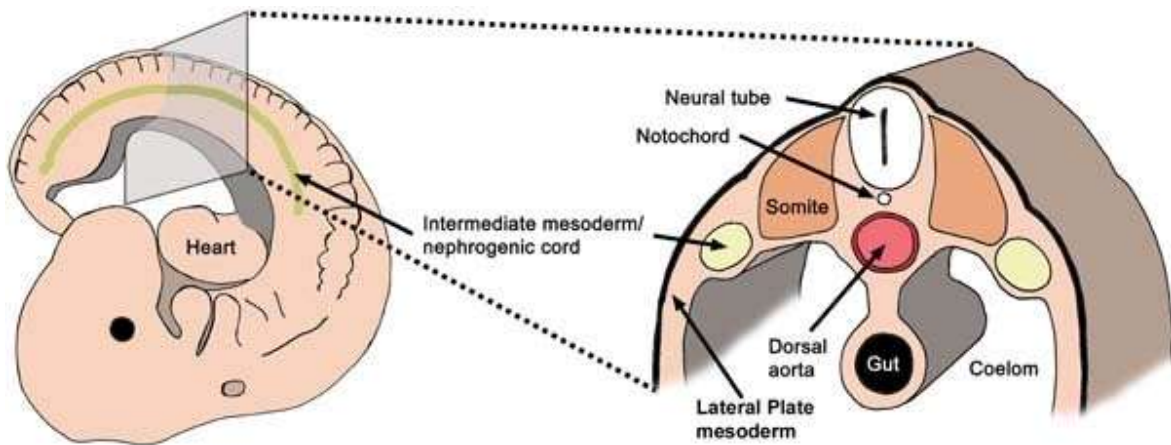


**Figure 1: Structure of the mammalian kidney.**

Each kidney is comprised of a fibrous outer layer called the renal capsule, a peripheral layer called the cortex, and an inner layer called the medulla. The medulla is arranged in multiple pyramidal structures that together with overlying cortex comprise a renal lobe (red box). Urine drains from the tip of each pyramid (papilla) into minor and major calices that empty into the renal pelvis. The renal pelvis then transmits the urine to the bladder via the ureter. Nephrons are found within the cortex and medulla and have a characteristic structure that includes a glomerular blood filter containing podocytes and a tubular epithelium that loops down into the medulla. The tubule is subdivided into proximal, intermediate, and distal segments (see color key) that are important for the recovery and modification of the glomerular filtrate.

Mouse kidney development begins with the formation of the pronephric duct (also known as the nephric or Wolffian duct; Figure 3). The nephric duct arises from the cranial portion of the intermediate mesoderm and grows caudally down the trunk to fuse with the urogenital sinus (ventral portion of the cloaca) at E11 (Hoar, 1976). As it migrates, the nephric duct induces the formation of mesonephric nephrons from the adjacent intermediate mesoderm (known as the nephrogenic cord or nephrogenic mesenchyme). Metanephros development is initiated at E10.5 at the caudal end of the nephric duct level with the hindlimb (27–28<sup>th</sup> somite). Glial-derived growth factor (GDNF), secreted from a unique population of nephrogenic cells called the metanephric mesenchyme, induces an outgrowth from the nephric duct called the ureteric bud (UB) that then invades the metanephric mesenchyme (see Figure 3). The UB forms a T-shaped bifurcation at E11.5, and then undergoes ~11 cycles of branching and elongation to generate the metanephric

collecting duct system (Cebrian et al., 2004). During this process, each UB tip is surrounded by a cap of metanephric mesenchyme, a subset of which give rise to nephron progenitors that proliferate, differentiate into glomerular and tubular epithelial cells, and fuse with the collecting duct. Early studies demonstrated that reciprocal and inductive signaling between the UB and the metanephric mesenchyme are essential for initiating and maintaining the cycles of UB branching and nephron induction that underlie the formation of the metanephros (Grobstein, 1955; Grobstein, 1956).

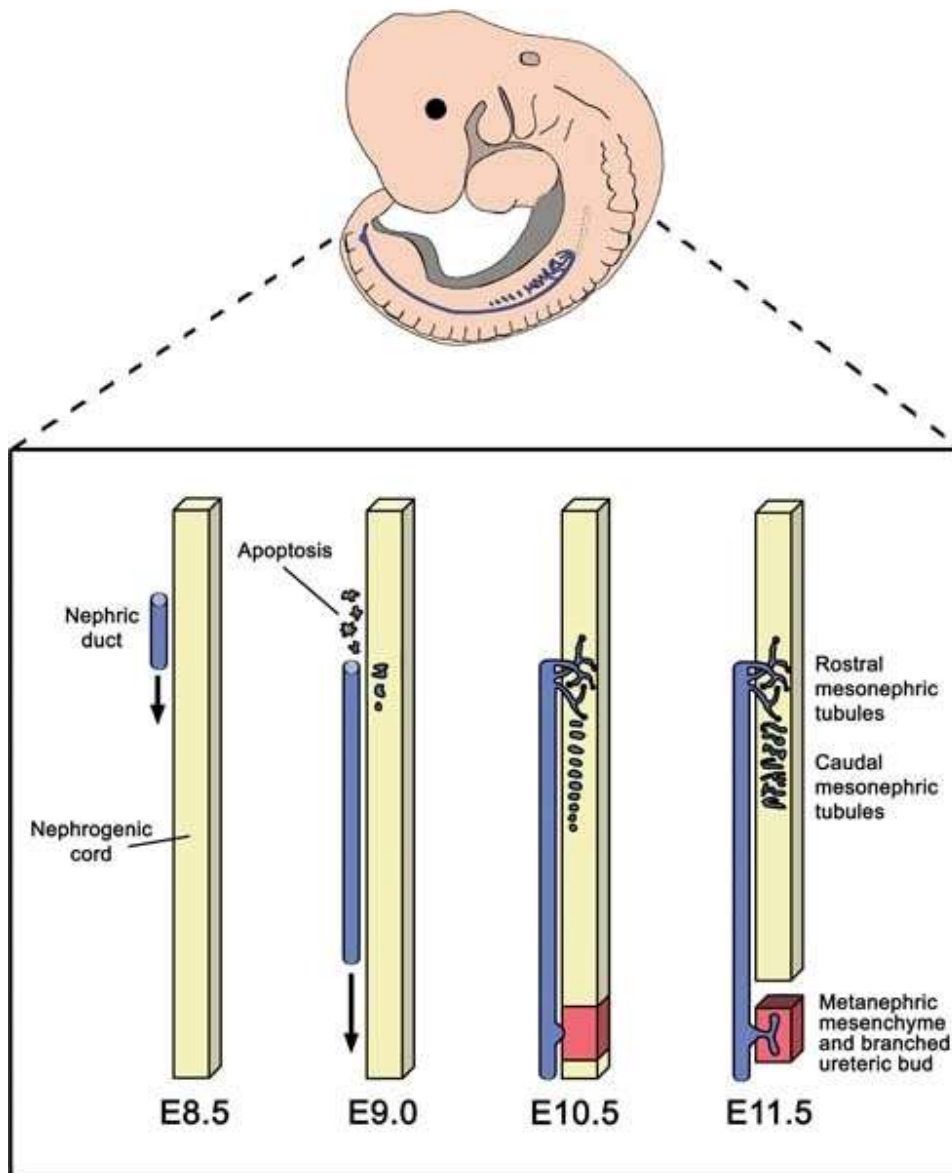


**Figure 2: Location of the intermediate mesoderm/nephrogenic cord.**

Schematic representation of a mouse embryo at E9.5 (left) and a cross-section through the trunk (right) showing the position of the intermediate mesoderm/nephrogenic cord.

### **Structure and function of the nephron**

The glomerulus is the most proximal component of the nephron and comprises a capillary tuft surrounded by highly specialized epithelial cells called podocytes (Quaggin and Kreidberg, 2008). Podocytes have a unique morphology that is characterized by several large primary projections, each of which is branched into smaller secondary extensions that branch again into a ‘comb-like’ array of ‘foot processes’. The foot processes from neighboring podocytes interdigitate, generating a series of narrow slits that are bridged by the slit diaphragm—a ‘protein zipper’ created by interactions between integral membrane proteins originating from adjacent foot processes. The foot processes are anchored to a specialized basal lamina, called the glomerular basement membrane (GBM), which also serves to separate the podocytes from the underlying endothelium. The endothelial cells of the glomerular capillary tuft are fenestrated, thus providing plasma solutes access to the GBM and slit diaphragm. Together, the fenestrated endothelium, GBM, and slit-diaphragm make up the glomerular filter and function to retain high molecular weight proteins and blood cells in the circulation while allowing small molecules such as water, sugars, and electrolytes to enter the nephron (Quaggin and Kreidberg, 2008).



**Figure 3: Overview of kidney development.**

Mouse kidney development begins at E8.5 with the formation of the nephric duct primordium (blue) from the nephrogenic cord (yellow). Rostral nephric duct precursors are lost by apoptosis while caudal cells persist and grow towards the cloaca. As it extends down the trunk the nephric duct induces the formation of mesonephric tubules in the adjacent nephrogenic cord. Only the rostral mesonephric tubules become joined to the nephric duct. At E10.5, an outgrowth called the ureteric bud forms from the nephric duct near the cloaca. The ureteric bud grows into a specialized population of nephrogenic cord cells (red) called the metanephric mesenchyme. Reciprocal inductive interactions between the ureteric bud and the metanephric mesenchyme lead to repeated branching of the ureteric bud and the formation of metanephric nephrons from the metanephric mesenchyme (not shown).

Once the filtrate has passed through the glomerular filter it encounters the first (proximal) portion of the tubule (see Figure 1). The proximal tubule is responsible for reabsorbing the majority of the filtrate, as it comprises metabolically useful solutes (Ullrich et al., 1963). The intermediate

and the distal segments of the tubule further fine-tune the composition of the filtrate and play an important role in regulating potassium, calcium, and magnesium homeostasis, as well as water absorption. To achieve these functions, each segment of the tubule has a distinct cellular morphology and expresses unique sets of solute transporters. Finally, the modified filtrate flows into the collecting duct, which in the case of the metanephric kidney, acts as the final regulator of electrolyte balance and water absorption (Hebert et al., 2001).

### **The intermediate mesoderm**

The mesoderm, together with the other two embryonic germ layers (ectoderm and endoderm), form during gastrulation. In general, gastrulation is characterized by the movement of an initially multi-potent population of epiblast cells into the interior of the embryo. In mammals, this occurs via the primitive streak, a furrow at the future caudal (posterior) end of the embryo where migrating epiblast cells undergo ingression (Gilbert, 2006). Once these cells have traversed the primitive streak they adopt either a mesodermal or endodermal fate and then become restricted to specific cell types within these lineages. This ‘patterning’ is best understood in fish and amphibian embryos, where signaling centers on opposite sides of the embryo (dorsal and ventral poles) release secreted factors, such as signaling molecules like the Bone Morphogenetic Proteins (BMPs) and their antagonists (reviewed by (De Robertis and Kuroda, 2004)). It is hypothesized that ‘dorsal versus ventral’ interactions, as well as positive and negative feedback loops occurring within each signaling center, establish morphogenic gradients across the gastrulating embryo. Particular mesodermal cell fates are thought to be induced, or ‘specified’, in response to high, medium, or low concentrations of these morphogens. Ultimately, dorsal-ventral patterning results in the mesoderm being subdivided into at least four major populations: (1) notochord (a transient ‘embryonic backbone’), (2) paraxial mesoderm (future somites ie progenitors of certain muscles and other connective tissues), (3) intermediate mesoderm (the precursor to the kidneys), and (4) lateral plate mesoderm (includes progenitors of the heart, blood, and vascular cells (see Figure 2)).

Perturbations in dorsal or ventral signaling pathways alter the proportions of these mesodermal populations within the embryo. ‘Dorsalized’ embryos have expanded notochord and paraxial mesoderm at the expense of intermediate and lateral plate mesoderm. Whereas ‘ventralized’ embryos have increased lateral plate and intermediate mesoderm at the expense of paraxial mesoderm and notochord. BMPs are potent ventralizing factors and there is considerable evidence from studies in *Xenopus*, zebrafish, and chicken to indicate that BMPs act during gastrulation to determine the size and location of the intermediate mesoderm along the medial-lateral axis of the embryo (James and Schultheiss, 2005; Kishimoto et al., 1997; Neave et al., 1997; Nguyen et al., 1998; Xu et al., 1999). BMPs are also needed after gastrulation to induce/maintain the expression of early acting pro-renal genes in the pronephros and to regulate various aspects of metanephric development (Bracken et al., 2008; Obara-Ishihara et al., 1999).

### **The pronephros and formation of the nephric duct**

The mouse pronephros arises from the portion of the intermediate mesoderm that is level with presumptive somites 5–8 at E8.0 (Bouchard et al., 2000; Vetter and Gibley, 1966). In this region, precursors of the pronephric duct separate away from the intermediate mesoderm and form a short longitudinal rod of cells that then grows caudally. These cells undergo a mesenchymal-to-epithelial transition and form the epithelial tube of the pronephric duct (also known as the nephric duct). At

the same time, ~3 small condensations of mesenchymal cells that may represent pronephric nephron precursors arise from the nephrogenic cord. However, these clusters do not differentiate further and pronephros development is aborted (Vetter and Gibley, 1966). The nephric duct degenerates by apoptosis soon after forming but persists in more caudal positions of the trunk where it is required for the formation of the mesonephric and metanephric kidneys (Pietila and Vainio, 2005; Pole et al., 2002).

### **Molecular regulation of nephric duct formation**

Early regulators of nephric duct development include the functionally redundant paired-box homeotic transcription factors Pax2 and Pax8, the zinc finger transcription factor Gata3, and the LIM class homeodomain transcription factor Lhx1 (previously known as Lim1).

*Pax2* and *Pax8* are co-expressed in nephric duct precursors at E8.5, although neither gene is dependent upon the other for their initial activation (Bouchard et al., 2002; Torres et al., 1995). *Pax2* and *Pax8* transcripts persist in the nephric duct as it extends caudally (and later in the metanephric collecting duct) and also appear in the nephrogenic mesenchyme of the mesonephros and metanephros where they play a role in nephron formation (see Section 6 below; Kobayashi et al., 2007; Narlis et al., 2007; Plachov et al., 1990; Torres et al., 1995). *Pax8* mutants do not display renal defects, whereas in *Pax2*-deficient mice, the nephric duct forms but does not reach the urogenital sinus and degenerates (Mansouri et al., 1998; Torres et al., 1995). In *Pax2/8* double mutants, there are no morphological signs of nephric duct formation and an absence of expression of the nephric duct markers *Ret*, *Lhx1*, and *Gata3* (Bouchard et al., 2002; Grote et al., 2006). As a result of failed nephric duct formation, no kidney types arise in the double mutants and the intermediate mesoderm undergoes apoptosis at E9.5 (Bouchard et al., 2002). These results indicate that Pax2 and Pax8 act redundantly at an early stage of renal development to commit intermediate mesoderm to the nephric duct lineage. This is not the sole function of Pax2 and Pax8 and other studies have identified additional roles including the formation of mesonephric and metanephric nephrons, UB outgrowth and branching during metanephros development, and anti-apoptotic activities in the metanephric mesenchyme, collecting duct, and nephrons (see Section 6 below for details).

*Gata3* is expressed in nephric duct precursors starting at E8.5 and is a putative direct target of Pax2 and Pax8 (Grote et al., 2006). Targeted inactivation of *Gata3* causes wayward growth of the nephric duct down the trunk and a loss of *Ret* expression. This defect leads to reduced mesonephric nephron formation and an absence of the metanephros due to a failure of the nephric duct to complete its extension to the urogenital sinus (Grote et al., 2006). These findings implicate Gata3 as a key regulator of guidance molecules that control nephric duct extension. Although *Ret* regulates chemotaxis in some cell types (see below), *Ret* null embryos do not display defects in nephric duct extension (Schuchardt et al., 1994) therefore other Gata3 targets must exist.

*Lhx1* is initially expressed in the intermediate mesoderm and lateral plate mesoderm at E7.5 and E8.5 but becomes restricted to the nephric duct primordium by E9.5 (Barnes et al., 1994; Fujii et al., 1994; Kobayashi et al., 2005; Tsang et al., 2000). Conventional as well as conditional gene targeting approaches have shown that *Lhx1* is required for the nephric duct to extend completely down the trunk and fuse with the urogenital sinus. The nephric duct undergoes necrotic degeneration, in *Lhx1* mutants possibly contributing to, or causing, the extension defect. Therefore, Lhx1 plays an important role in nephric duct survival. These defects lead to corresponding abnormalities in the formation of the mesonephric and metanephric kidneys with the most severe

phenotype being metanephric agenesis (Kobayashi et al., 2005; Pedersen et al., 2005; Shawlot and Behringer, 1995). Direct targets of *Lhx1* in the nephric duct are not known and *Pax2* expression is not dependent on *Lhx1* function (Kobayashi et al., 2005; Pedersen et al., 2005; Tsang et al., 2000). Transcripts for *E-cadherin* and *Wnt9b* (implicated in mesonephric and metanephric nephron induction, see below) are reduced in *Lhx1* mutants but it is not clear if this is secondary to cell death (Pedersen et al., 2005). *Lhx1* is also expressed in nascent nephrons during mesonephric and metanephric kidney development and like *Pax2/8*, is required at multiple steps of kidney development.

## The Mesonephros

The mouse mesonephros consists of up to ~18 pairs of tubules that extend from the level of somite 10 to 17 and are divided into distinct cranial and caudal sets (Sainio, 2003; Vetter and Gibley, 1966). The nephrons of the cranial set develop rudimentary glomeruli, are frequently branched, and join with the nephric duct at 4–6 sites. Based on the observation that the cranial nephrons appear to be in continuous contact with the nephric duct during their development it has been suggested that the cranial set may form directly from the nephric duct, perhaps as outgrowths (Sainio et al., 1997). However, data from chicken and mouse studies indicate that the duct only contributes cells to a short connecting segment (Brenner-Anantharam et al., 2007; Croisille et al., 1976; Mugford et al., 2008). In contrast, the caudal pairs, which make up the bulk of the mesonephros, are comprised of primitive unbranched tubules that do not connect to the nephric duct and derive from the nephrogenic cord (see Figure 2). These tubules first appear as condensations of nephrogenic cord cells level with somites 8–9 at E9.0 (Vetter and Gibley, 1966). These cells undergo a mesenchymal-to-epithelial transition, initially forming a ‘renal vesicle’ and then elongating into a ‘S-shaped body’ (Smith and Mackay, 1991). The mesonephros begins degenerating at E14.5 and within 24 hours almost all of the tubules undergo apoptosis and disappear in a caudal to cranial direction (Sainio et al., 1997; Smith and Mackay, 1991). In females, all of the tubules are lost, whereas in males some of the cranial tubules remain intact, contributing to the epididymal ducts of the testis (Vetter and Gibley, 1966).

The molecular regulation of mouse mesonephros development has received little attention, presumably because of the limited functionality and transient existence of the organ. Classical experiments in chick embryos have demonstrated that microsurgical disruption of nephric duct extension results in a failure of mesonephric nephron formation in the adjacent nephrogenic cord (Boyden, 1927; Waddington, 1938). Thus, it has long been suspected that nephron-inducing signals emanate from the nephric duct as it migrates to the cloaca, resulting in the formation of mesonephric nephrons in a craniocaudal sequence. The gene responsible for nephron induction was recently identified as *Wnt9b*, encoding a member of the Wnt family of secreted signaling molecules that is expressed by the nephric duct during mouse mesonephric and metanephric development (Carroll et al., 2005). *Wnt9b* mutants also lack metanephric nephrons (see below), suggesting that similar nephron-inducing programs underlie nephron formation in both mesonephric and metanephric kidneys. In support of this, the transcription factors *Pax2*, Wilms’ tumor suppressor1 (*Wt1*), *Foxc1*, and *Six1*, which are involved in metanephros formation (see below), are also involved in mesonephric nephron formation (Note: *Wt1* and *Six1* are reported to be only required for the formation of the caudal set of nephrons, however the cranial tubules seen in these mutants may actually represent arrested nephric duct outgrowths; (Kobayashi et al., 2007; Mugford et al., 2008; Sainio et al., 1997; Torres et al., 1995).



## **The Metanephros**

Our understanding of the developmental pathways underlying metanephros formation has advanced significantly over the last two decades. The metanephric mesenchyme expresses a large number of regulatory genes, including the transcriptional regulators *Pax2*, *Wt1*, *Eya1*, *Six1*, *Six2*, *Osr1*, and *Hox11*. These factors function as regulators of the formation, proliferation, and survival of the metanephric mesenchyme and/or as upstream activators of *Gdnf*.

### **Specification of the metanephric mesenchyme**

The nephric duct is not required for the initial specification of the metanephric mesenchyme as in the *Gata3* knockout mouse, *Pax2*-expressing metanephric mesenchyme is still found despite failure of the nephric duct to migrate to this region of the embryo (Grote et al., 2006). Early key factors that have been identified as being necessary for the formation of a morphologically distinct region of metanephric mesenchyme are the transcriptional regulators *Eya1*, *Six1/Six4*, and *Odd1*.

### **Eyes-absent-1 (Eya) and Sine-Oculis (Six)**

*Eya1*, a homolog of *Drosophila eyes absent*, encodes a transcriptional co-activator that can complex with other transcription factors to induce the expression of target genes (Jemc and Rebay, 2007). At E8.5, *Eya1* is expressed in the nephrogenic cord caudal to the mesonephros but gets progressively restricted until it is only expressed by the metanephric mesenchyme at E11.5 (Sajithlal et al., 2005). *Eya1*-deficient embryos do not form a morphologically distinct population of metanephric mesenchyme or a UB at E10.5 (Xu et al., 1999). *Eya1* null embryos display an early loss of *Gdnf* expression in the nephrogenic cord (from E9.5 onwards). This defect is not caused by a failure in the formation of the intermediate mesoderm, as normal expression of *Wt1* and *Pax2* is observed in the nephrogenic cord at E9.5 (Sajithlal et al., 2005; Xu et al., 1999). At E10.5, transcripts for *Pax2* and *Six2* are absent in the metanephric region of *Eya1* null embryos, suggesting that the metanephric mesenchyme fails to be specified in the mutants. In addition, significant apoptosis in the metanephric region is also observed in the mutants at this stage (Sajithlal et al., 2005; Xu et al., 1999). Although an absence of metanephric mesenchyme marker expression could be secondary to cell loss, a more recent analysis of *Eya1* hypomorphic mutants has provided further evidence to support an early role for *Eya1* during metanephric mesenchyme differentiation. *Eya1* hypomorphs with ~20% of normal *Eya1* protein levels initially develop both the metanephric mesenchyme and a UB, although further UB outgrowth and branching is defective (Sajithlal et al., 2005). Expression of *Pax2*, *Six1*, and *Gdnf* in the metanephric mesenchyme is reduced in the hypomorphs at E10.5, suggesting that *Eya1* is required to induce or maintain the expression of these genes. Taken together, these results suggest that *Eya1* acts during the early stages of metanephric kidney development to convert nephrogenic cord cells into metanephric mesenchyme, at least in part, by regulating the expression of *Pax2*, *Six1*, *Six2*, and *Gdnf*.

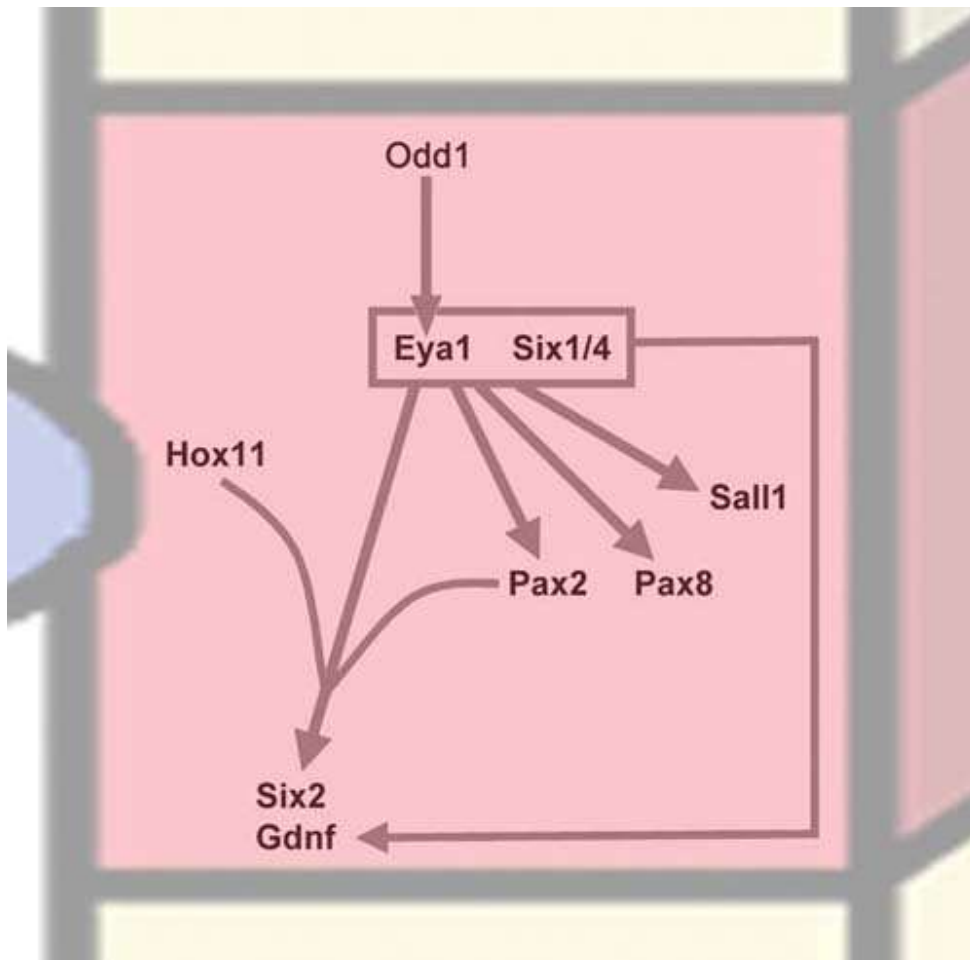
*Eya1* has no apparent DNA-binding activity and is translocated from the cytoplasm to the nucleus by binding to the Six (sine oculis) homeobox transcription factors (Ohto et al., 1999). Candidate Six proteins involved in renal development include *Six1*, *Six2*, and *Six4*, which show overlapping expression patterns in the nephrogenic cord and metanephric mesenchyme (Kobayashi et al., 2007; Li et al., 2003; Oliver et al., 1995; Xu et al., 2003). Functionally, the Six



proteins may share similar or redundant activities. Consistent with this, no single knock-out of a *Six* gene recapitulates the severity of the *Eya1* null phenotype. In *Six1* null embryos, the metanephric mesenchyme is specified, as determined by morphology and the expression of *Wt1* and *Eya1*, but its size is reduced (Li et al., 2003). Expression of the metanephric mesenchyme markers *Sall1*, *Pax2*, and *Six2* are severely reduced in the mutants, although differing degrees of down-regulation have been reported (Kobayashi et al., 2007; Li et al., 2003; Sajithlal et al., 2005; Xu et al., 2003). *Gdnf* is normally expressed in the metanephric mesenchyme of *Six1* mutants, although its overall expression domain is smaller given the reduced size of the metanephric mesenchyme (Xu et al., 2003). The UB initially forms at E10.5 in *Six1* mutants but often fails to invade the metanephric mesenchyme, perhaps due to a reduced local concentration of *Gdnf* caused by the smaller mass of metanephric mesenchyme (Yu et al., 2004). As a result, apoptosis of the metanephric mesenchyme ensues at E11.5 and renal agenesis is seen in the majority of *Six1*-null newborns (Kobayashi et al., 2007; Xu et al., 2003).

*Six2* null embryos do not show defects in the initial specification of the metanephric mesenchyme or UB outgrowth, despite expression of *Six2* in the metanephric mesenchyme at E10 and the finding that *Six2* can activate *Gdnf* expression (Brodbeck et al., 2004; Self et al., 2006). However, later abnormalities in early nephron formation are found at E11.5 (see below). *Six1/Six2* double mutants have not yet been reported. *Six4*-deficient mice are viable and fertile and exhibit no major developmental defects (Ozaki et al., 2001). A recent analysis of *Six1/Six4*-deficient embryos has demonstrated clear functional redundancy between the *Six1* and *Six4* proteins with loss of both genes leading to a more severe metanephric phenotype than that of *Six1*-deficient mutants (Kobayashi et al., 2007). A morphologically distinct region of metanephric mesenchyme is missing in *Six1/Six4* double mutants and there is an absence of *Pax2*, *Sall1*, and *Gdnf* expression in the metanephric region. In addition, *Pax8*, which functions redundantly with *Pax2* (see above), is transiently expressed in the metanephric mesenchyme at E10.0 and while it is normally expressed in *Six1*-deficient embryos, it is absent in the *Six1/Six4* double mutants. Nephrogenic cells appear to still be present in the *Six1/Six4* mutants based on the expression of *Wt1*, *Six1*, and *Osr1* (Kobayashi et al., 2007). No apoptosis in the metanephric region was observed at E10.5, although it presumably occurs at least as early as E11.5 based on the *Six1* phenotype (see above). The UB fails to form in *Six1/Six4* mutants and renal agenesis is seen in all of the newborns.

Overall, the phenotype of *Six1/Six4* mutants is similar to that of *Eya1* mutants, strongly suggesting that *Six1* and *Six4* interact redundantly with *Eya1* to activate expression of metanephric regulators such as *Pax2/8*, *Six2*, *Sall1*, and *Gdnf*. Whether *Eya1/Six* complexes directly activate the expression of these genes is not clear, although chromatin immunoprecipitation experiments have shown that *Eya1* and *Six1* are present on *Gdnf* regulatory sequences (Li et al., 2003). *Eya1* can interact with other transcription factors and a recent study has implicated an *Eya1-Pax2-Hox11* complex as a direct activator of *Six2* as well as *Gdnf* (Gong et al., 2007; Mugford et al., 2008). Consistent with this, targeted inactivation of all three *Hox11* paralogs causes a significant reduction in *Six2* and *Gdnf* expression in the metanephric mesenchyme at E10.5 (Wellik et al., 2002). However, expression of *Pax2* is normal in *Hox11* triple mutants whereas it is absent in *Six1/Six4* double mutants, suggesting that a *Hox11-Eya1-Pax2* complex may act later in development, perhaps after an *Eya1/Six1/4* complex has upregulated *Pax2* and *Sall1* in the metanephric mesenchyme (see Figure 4). The ability of *Eya1* to interact in multiple complexes and to induce the expression of genes encoding transcription factors it physically associates with, make it difficult to place *Eya1* in a simple linear pathway.



**Figure 4A: Model of potential epistatic interactions occurring between early-acting transcription factors in the nephrogenic cord at the level of the presumptive metanephros.**

### Odd-skipped related-1

*Odd-skipped related-1 (Odd1)* encodes a zinc-finger transcription factor related to the *Drosophila* pair rule gene *odd skipped* (Coulter et al., 1990). *Odd1* is one of the earliest molecular markers of the intermediate mesoderm in the mouse embryo and is first activated in these cells shortly after they migrate from the primitive streak at the midgastrula stage (Wang et al., 2005). At E8.5–9.5, *Odd1* transcripts are found throughout the intermediate mesoderm/nephrogenic cord and in the metanephric mesenchyme but not the UB at E10.5 (James et al., 2006; So and Danielian, 1999; Wang et al., 2005). In *Odd1* null embryos, neither the UB nor a morphologically distinct population of metanephric mesenchyme can be detected and renal agenesis occurs in mutants that survive to late gestation (Wang et al., 2005). Consistent with this, *Odd1* mutants completely lack *Eya1* and *Pax2* in the metanephric region at E9.5 (James et al., 2006). *Wt1* expression levels are reduced in the *Odd1* mutants, but *Wt1* protein can still be detected in the metanephric region. High levels of apoptosis are evident in the mutant metanephric mesenchyme at E10.5, consistent with

the downregulation of *Eya1* (see above; James et al., 2006; Wang et al., 2005). At E11.5, transcripts for *Six2*, *Gdnf*, and *Sall1* cannot be detected, also possibly due to the absence of *Eya1*, however cell loss by apoptosis may contribute to the failure to detect markers of the metanephric mesenchyme.

*Odd1* mutants also display nephric duct defects that are characterized by discontinuous expression of *Ret*, *Lhx1*, *Pax2*, posterior truncations, and a smaller nephric duct diameter (James et al., 2006; Wang et al., 2005). The cause of these abnormalities has not been examined but may be due to increased nephric duct cell death, perhaps indirectly resulting from the loss of the adjacent nephrogenic cord (given that *Odd1* transcripts are not found in the migrating nephric duct; James et al., 2006). Interestingly, the duct defects are more severe on the left side of the embryo, suggesting that pathways that establish left-right patterning may interact with *Odd1* (Wang et al., 2005).

The direct targets of *Odd1* during kidney development are not known and current evidence indicates that *Odd* family members act as transcriptional repressors (Goldstein et al., 2005; Tena et al., 2007). Thus, the effects of *Odd1* on renal genes such as *Eya1* and *Pax2* may be mediated indirectly via the inhibition of a repressor of these genes. Taken together, these findings place *Odd1* as one of the earliest acting genes involved in metanephros formation and suggest that *Odd1* acts upstream *Eya1*, *Pax2*, and *Wtl* in the nephrogenic cord to promote metanephric mesenchyme formation and survival (see Figure 4 and Figure 5).

### **Paired-box transcription factor (Pax) 2 and Pax8**

*Pax2* is expressed in the nephrogenic cord in the metanephric region at E9.5 and then in the metanephric mesenchyme at E10.0. Transcripts for *Pax2* are also found in the nephric duct (as described above) and in the UB as it invades the metanephric mesenchyme (Brophy et al., 2001; Torres et al., 1995). In *Pax2* mutants, the nephric duct does not reach the cloaca, expression of *Ret* is lost, and the duct starts degenerating by E12.5. As a result, the UB never forms and metanephric development does not occur (Torres et al., 1995). The metanephric mesenchyme can be morphologically distinguished in *Pax2* mutants and expresses *Eya1*, *Six1*, *Six2* (albeit weakly) and *Sall1*, indicating that *Pax2* is not required for the initial specification of the metanephric mesenchyme from the nephrogenic cord (Torres et al., 1995; Xu et al., 2003). However, the transient expression of *Pax8* in the metanephric mesenchyme at E10.0 may mask an earlier role for *Pax2* and *Pax8* in the formation of the metanephric mesenchyme (Kobayashi et al., 2007). Transcripts for *Gdnf* are reduced in the *Pax2*-deficient metanephric mesenchyme, consistent with *Pax2* participating in a transcriptional complex with *Eya1* and *Hox11* proteins on *Gdnf* regulatory sequences (see above; (Sajithlal et al., 2005).

### **Ureteric Bud outgrowth**

#### **Glial-derived neurotrophic factor**

*Gdnf* is expressed broadly throughout the nephrogenic cord at E9.5 but becomes restricted to the region of the metanephric mesenchyme by E10.5 (Grieshammer et al., 2004; Hellmich et al., 1996; Pichel et al., 1996; Sanchez et al., 1996). GDNF signals through the *Ret* tyrosine kinase receptor, which is expressed by the nephric duct, together with a membrane-tethered *GFRa1* co-receptor (Baloh et al., 1997; Pachnis et al., 1993; Sainio et al., 1997). GDNF signaling also requires cell surface heparan sulphate glycosaminoglycans that bind to GDNF and may play a role in ligand

presentation to the receptor (Bullock et al., 1998; Davies et al., 2003; Rider, 2003; Tanaka et al., 2002). GDNF binding to the Ret receptor leads to activation of the phosphatidyl inositol 3-kinase and extracellular signal-related kinase (ERK) signaling pathways and promotes chemotaxis and proliferation in a range of cells (Fisher et al., 2001; Hu et al., 1999; Natarajan et al., 2002; Tang et al., 2002; Tang et al., 1998; Watanabe and Costantini, 2004; Young et al., 2001). The majority of mouse embryos deficient in *Gdnf*, *Ret*, or *Gfra1* fail to form a UB, although the observation that a UB forms in a fraction of the mutants indicates that additional UB inducing signals must also exist (Cacalano et al., 1998; Maeshima et al., 2007; Moore et al., 1996; Pichel et al., 1996; Sanchez et al., 1996).

Various studies using GDNF-soaked beads and transgenic overexpression of *Gdnf* have demonstrated the importance of localizing GDNF-Ret signaling so as to prevent the formation of ectopic UBs (Brophy et al., 2001; Sainio et al., 1997). The maintenance and/or activation of *Gdnf* expression in the metanephric mesenchyme are dependent upon a host of regulatory factors including transcription factors (*Eya1*, *Six1*, *Sall1*, *Pax2*, *Hox11* proteins), secreted factors (*Gdf11*, *Nephronectin*) and FGF signaling (see above and below for details). Inhibitors of *Gdnf* expression include *Foxc1*, a forkhead transcription factor, and the *Robo2*/*Slit2* receptor/ligand pair best known for their chemorepellent role during neuron and axon migration (Andrews et al., 2007). In embryos deficient in *Foxc1* there is an abnormal maintenance of *Gdnf* (and *Eya1*) in more anterior portions of the nephrogenic cord and a corresponding induction of ectopic UB from portions of the nephric duct just anterior to the normal site of UB formation (Kume et al., 2000). Similar phenotypes of expanded *Gdnf* expression and supernumerary UBs are seen in *Slit2* and *Robo2* mutants (Grieshammer et al., 2004). *Slit2* is expressed in the nephric duct and weakly in the metanephric mesenchyme, with higher levels in the nephrogenic cord anterior the *Gdnf* expression domain. *Robo2* is highly expressed in the nephrogenic cord but weakly in the region of the nephric duct where the UB forms. Thus, the expression patterns of *Slit2* and *Robo2* are consistent with either a role in restricting *Gdnf* expression or possibly a more general role in restricting the size of the metanephric mesenchyme. The downstream targets of *Slit2*-*Robo2* signaling in the nephrogenic cord are unknown but do not appear to be mediated via alterations in the expression domains of *Foxc1* or *Eya1* (Grieshammer et al., 2004).

### **Fibroblast growth factors**

The first indication that signaling via the FGF receptors (FGFRs) was important for early metanephric development came from the study of a transgenic line that expressed a secreted dominant negative FGF receptor that could inhibit multiple FGFs (Celli et al., 1998). Half of the mutant transgenic embryos at E18.5 displayed renal agenesis or small malformed kidneys. A more definitive requirement of FGF signaling for UB outgrowth came from the conditional inactivation of *Fgfr1* and *Fgfr2* (Poladia et al., 2006). These FGF receptors are expressed in both the metanephric mesenchyme and UB at E10.5 and E11.5. While single deletion of *Fgfr1* or *Fgfr2* from the metanephric mesenchyme does not result in any kidney abnormalities, loss of both receptors causes renal agenesis (Poladia et al., 2006). The UB initially forms normally in the double mutants at E10.5 but it does not elongate further or branch and undergoes apoptosis by E11.5. Interestingly, *Fgfr1/2* mutants occasionally develop two UBs, suggesting that FGF signaling in the metanephric mesenchyme is also needed to prevent ectopic UB outgrowth. The metanephric mesenchyme is greatly reduced in *Fgfr1/2* mutants and appears hypo-proliferative and apoptotic at E10.5. Despite this, *Gdnf* is still expressed at this stage, consistent with the initial

formation of the UB in the mutants. Subsequently, *Gdnf* levels are lost at E11.5 suggesting that a failure to maintain *Gdnf* expression is the underlying cause for the arrested UB outgrowth phenotype. The *Fgfr1/2* deficient metanephric mesenchyme, albeit reduced in size, expresses *Eya1*, *Six1*, and *Wt1* at E10.5. However, transcripts for *Pax2*, *Six2*, and *Sall1* are absent in the mutants at this stage, indicating that FGF signaling lies upstream of these transcription factors. The FGF ligand/s responsible for activating FGFR1 or FGFR2 in the metanephric mesenchyme are not known and no single *Fgf* knockout has recapitulated the *Fgfr1/r2*-deficient renal phenotype, presumably due to redundancy.

## **Sall1**

The *Sall* gene family in mammals includes four members (*Sall1–4*) that encode homologues of the *Drosophila* homeotic gene *Spalt*, a multi-zinc finger transcription factor that acts as both a repressor and an activator (Nishinakamura and Osafune, 2006). Of these *Sall1* plays a clear role in the development of the metanephros. Transcripts for *Sall1* are found in the metanephric mesenchyme at E10.5, prior to UB formation. In *Sall1*-null embryos, the metanephric mesenchyme can be morphologically distinguished, although it is reduced in size, and expresses a number of molecular markers such as *Pax2*, *WT1*, and *Eya1*. The UB forms in *Sall1*-deficient embryos but it does not complete its outgrowth into the metanephric mesenchyme and renal agenesis occurs in a high percentage of the newborns (Nishinakamura et al., 2001). As with other mutants that show failed UB invasion, expression of *Gdnf* is initially normal in *Sall1* mutants at E10.5 but is lost by E11.5. Similarly, increased apoptosis of the metanephric mesenchyme, a hallmark of failed UB outgrowth, is evident in *Sall1* mutants at E11.5. *Sall1/Sall4* compound heterozygotes have also been reported to display a renal agenesis phenotype, however the expression pattern of *Sall4* in the metanephros has not been described and the details of this genetic interaction are unclear (Sakaki-Yumoto et al., 2006).

Expression of *Sall1* is absent in *Six1* mutants suggesting that *Sall1* may be a target of an Eya1-Six1-containing transcriptional complex (see Figure 4). Consistent with this, analysis of the human *SALL1* promoter identified Six1 binding sites and demonstrated synergistic activation of the promoter *in vitro* following *Six1* and *Eya1* transfection (Chai et al., 2006). *Sall1* and *Six1* mutants have common renal defects including reduced size of the metanephric mesenchyme and failed UB invasion (Li et al., 2003; Xu et al., 2003). *Sall1* expression is also lost in *Fgfr1/2* conditional mutants (see above), indicating that both FGF signaling and an Eya1-Six1 complex is necessary for *Sall1* expression in the metanephric mesenchyme.

## **Growth/differentiation factor 11**

A loss of *Gdnf* expression in the metanephric mesenchyme is seen at E11.5 in mutants defective in *Growth/differentiation factor 11* (*Gdf11*), encoding a member of the TGF- $\beta$ /BMP superfamily. *Gdf11* is expressed in the nephric duct, UB, and the metanephric mesenchyme at E10.5 (Esquela and Lee, 2003). The majority of *Gdf11* mutants show bilateral renal agenesis resulting from an absence of UB outgrowth, presumably due to the loss of *Gdnf* expression in the metanephric mesenchyme. Thus, GDF11 is implicated as an upstream inducer of *Gdnf* expression. However, earlier stages were not examined in the *Gdf11* mutants and it is not known if *Gdnf* is initially expressed at E10.5 but then is not maintained in the metanephric mesenchyme at later stages. Maintenance of *Gdnf* expression in the metanephric mesenchyme after E10.5 depends upon

UB invasion of the metanephric mesenchyme and most likely the establishment of a GDNF-Ret positive feedback loop (see below). Therefore, the reduced expression of *Gdnf* in *Gdf11* mutants could be a secondary consequence of failed UB invasion.

GDF11 can signal via complexes of the Activin type 2b receptor (Acvr2b) and the ALK5 type 1 receptor, and consistent with this, renal agenesis is seen in *Alk5*<sup>+/-</sup>; *Acvr2b*<sup>-/-</sup> mutants (Andersson et al., 2006). GDF11 signaling via Alk5/Acvr2b has also been implicated in anterior-posterior patterning of the embryonic axis (upstream of the *Hox* genes) and the hindlimbs and metanephric mesenchyme are posteriorly displaced in the mutants (Andersson et al., 2006; McPherron et al., 1999). Whether axial patterning abnormalities are involved in the UB outgrowth defect is not known but the metanephric mesenchyme appears to form normally based on its morphological appearance, expression of markers such *Eya1* and *Pax2*, and its ability to undergo nephrogenesis *in vitro* (Esquela and Lee, 2003). The nephric duct from *Gdf11* mutants can be induced in culture to form UB outgrowths in response to exogenous GDNF, consistent with the notion that the primary defect is a loss of *Gdnf* expression in the metanephric mesenchyme. Interestingly, in these experiments it was found that the *Gdf11* mutant nephric duct is hypersensitive to GDNF, thus raising the possibility that GDF11 may function to antagonize inhibitors of UB outgrowth (see below).

### **Nephronectin and $\alpha 8 \beta 1$ integrin**

*Gdnf* expression in the metanephric mesenchyme is transiently dependent on the extracellular matrix (ECM) protein Nephronectin (Npnt) and the cell adhesion receptor subunit  $\alpha 8$  integrin (Itga8; Brandenberger et al., 2001; Linton et al., 2007). The  $\alpha 8$  integrin subunit forms a dimeric cell surface receptor complex with  $\beta 1$  integrin ( $\alpha 8 \beta 1$ ) and binds a number of ECM proteins including Nephronectin. Transcripts for *Itga8* are found throughout the nephrogenic cord as well as the metanephric mesenchyme, while *Npnt* is expressed by the UB (Brandenberger et al., 2001; Muller et al., 1997). The renal phenotypes of *Npnt* and *Itga8* null mutants are very similar; the UB initially forms but its invasion into the adjacent metanephric mesenchyme is delayed (Linton et al., 2007; Muller et al., 1997). Although some recovery in UB growth and branching subsequently occurs, this is not sufficient to prevent a high frequency of kidney agenesis occurring in the newborn mutants. This defect is correlated with a transient down-regulation of *Gdnf* transcripts in the metanephric mesenchyme at E11.5, followed by restored expression at E13.5, and normal levels of *Gdnf* regulators such as *Eya1*, *Pax2*, *Six2*, and *Gdf11* (Linton et al., 2007). The penetrance of the *Itga8* mutant kidney phenotype can be made more or less severe by genetically modulating the level of GDNF-Ret signaling *in vivo*, supporting the hypothesis that a transient reduction in *Gdnf* expression underlies the cause of the kidney agenesis phenotype (Linton et al., 2007).

### **Wilms' Tumor Suppressor-1**

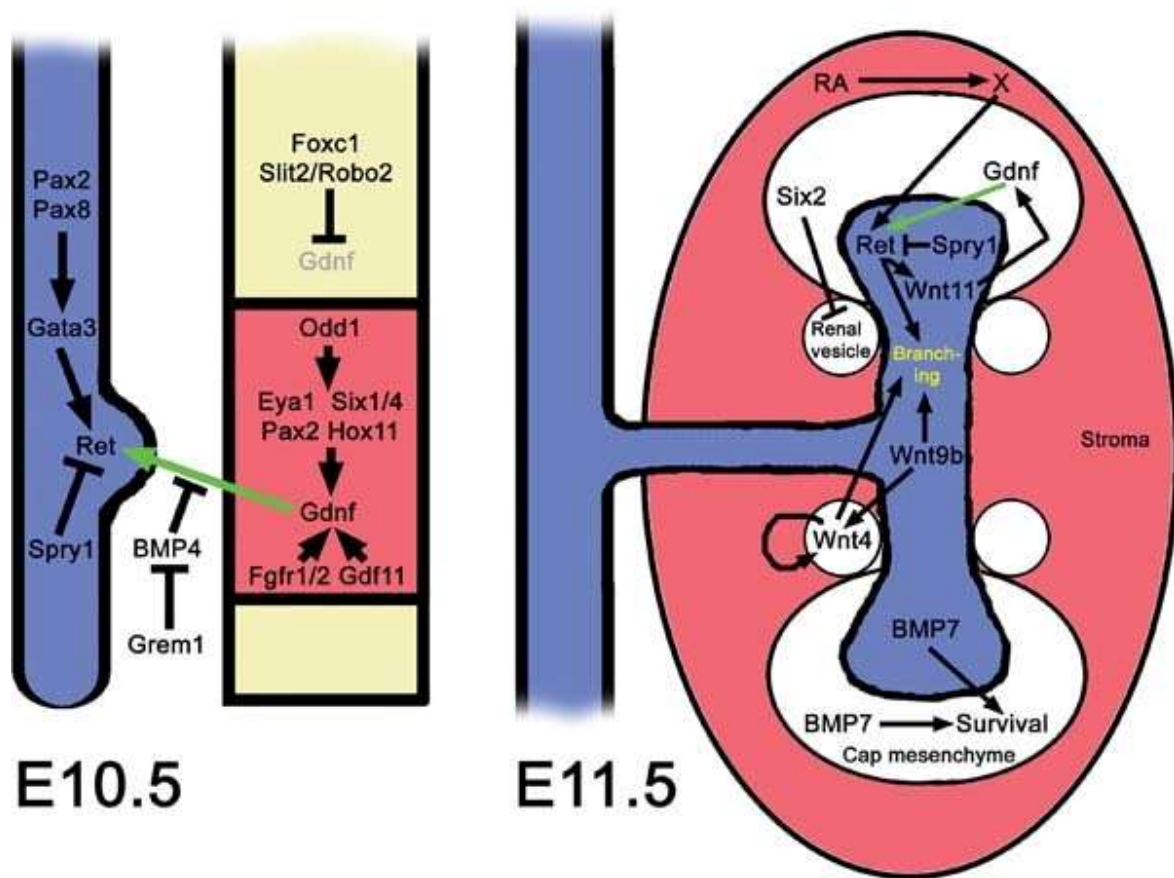
The *Wilms' tumor suppressor-1* gene (*Wt1*) encodes a zinc finger transcription factor and mRNA splicing co-factor. WT1 is considered a potential regulator of GDNF signaling, although the details of how WT1 interacts with the GDNF pathway remain obscure. *Wt1* is initially expressed throughout the nephrogenic cord as early as E9.0 and is maintained weakly in the metanephric mesenchyme prior to UB outgrowth at E10.5 (Armstrong et al., 1993; Pellegrini et al., 1997). The UB fails to form in *Wt1* null mutants and the metanephric mesenchyme, which appears morphologically normal and expresses *Pax2* and *Six2*, undergoes apoptosis at E11.5 and

is lost by E12.0 (Donovan et al., 1999; Kreidberg et al., 1993). Although this phenotype resembles loss of GDNF signaling, transcripts for *Gdnf* are still detectable in the metanephric mesenchyme of *Wt1* mutant embryos (Donovan et al., 1999). This finding led to the suggestion that GDNF protein levels may be reduced or absent in *Wt1* mutants or alternatively *Wt1* may be needed to boost *Gdnf* expression levels above a critical threshold needed for UB outgrowth (Donovan et al., 1999; Gao et al., 2005). A more recent study suggests that *Wt1* may act upstream of *vascular endothelial growth factor-a* (*Vegfa*) to stimulate angioblasts to release an, as-of-yet unidentified, factor that induces *Pax2* and *Gdnf* expression. *WT1* is likely to play multiple roles during metanephros development as classic tissue recombinant experiments have also shown that *Wt1* mutant metanephric mesenchyme can not be induced to undergo nephrogenesis (Donovan et al., 1999; Kreidberg et al., 1993).

### **Sprouty1**

Supernumerary UB buds that are not associated with alterations in the *Gdnf* expression domain are seen in embryos deficient in *Sprouty1* (*Spry1*; Basson et al., 2005). *Sprouty* genes encode cytoplasmic membrane-associated inhibitors of receptor tyrosine kinase signaling (Mason et al., 2006). *Spry1* is expressed in both the nephric duct and the metanephric mesenchyme, however, conditional inactivation of *Spry1* in the nephric duct results in a similar phenotype to the conventional null mutant (Basson et al., 2005). Therefore, *Spry1* function is required in the nephric duct to prevent ectopic UB formation, most likely by preventing excessive GDNF-Ret signaling. In support of this role, reducing the level of GDNF in *Spry1* null embryos by inactivating one *Gdnf* allele rescued the kidney defects in 75% of the compound mutants. Expression of *Spry1* in the nephric duct was upregulated in response to GDNF-soaked beads and reduced in *Ret* mutant embryos, consistent with the observation that *Sprouty* genes are often upregulated by the same pathways that they inhibit (Basson et al., 2005; Mason et al., 2006). Based on these findings, *Spry1* likely forms part of a GDNF-Ret negative feedback loop that lowers the sensitivity of the nephric duct to GDNF and helps ensure that only a single UB is induced during metanephros development (see Figure 5).





**Figure 5: Key molecular pathways involved in early metanephric kidney development.**

At E10.5, the metanephric mesenchyme (red) comprises a unique subpopulation of the nephrogenic cord (yellow). Expression of the *Glial-derived neurotrophic factor* (*Gdnf*) is restricted to the metanephric mesenchyme by the actions of transcriptional activators, secreted factors, and inhibitors. GDNF binds the Ret receptor and promotes the formation of the ureteric bud, an outgrowth from the nephric duct (blue). Ret initially depends upon the Gata3 transcription factor for its expression in the nephric duct. Spry1 acts as an intracellular inhibitor of the Ret signal transduction pathway. BMP4 inhibits GDNF signaling and is in turn inhibited by the Grem1 binding protein. At 11.5, the ureteric bud has branched, forming a T-shaped structure. Each ureteric bud tip is surrounded by a cap of condensed metanephric mesenchyme. Reciprocal signaling between the cap mesenchyme and ureteric bud, as well as signals coming from stromal cells (red), maintain expression of *Ret* in the bud tips and *Gdnf* in the cap mesenchyme. Nephrons are derived from cap mesenchyme cells that form pretubular aggregates and then renal vesicles on either side of each ureteric bud tip. Wnt9b and Wnt4 induce nephron formation and are necessary for maintaining ureteric bud branching. The Six2 transcription factor prevents ectopic nephron formation. BMP7 promotes survival of the cap mesenchyme. Not all genes implicated in metanephros formation are shown for clarity (see text for further details). Green arrows indicate the ligand-receptor interaction between GDNF and Ret. Black arrows indicate the epistasis between genes but in most cases it is not known if the interactions are direct. T-shaped symbols indicate inhibitory interactions.

### Bone morphogenetic protein 4 and gremlin1

BMP4 has also been implicated as a negative regulator of UB outgrowth. BMP4 is expressed in stromal cells enveloping the nephric duct prior to the outgrowth of the UB (Dudley and Robertson, 1997). Although *Bmp4* null embryos die during early development, heterozygotes frequently display an ectopic UB (Miyazaki et al., 2000; Miyazaki et al., 2003; Winnier et al.,

1995). Organ culture studies have revealed that BMP4 can block the ability of GDNF to induce ectopic budding from the nephric duct (Brophy et al., 2001). BMP4 activity can be inhibited following binding to the secreted BMP antagonist encoded by *Gremlin1* (*Grem1*), which is expressed in an overlapping expression domain with *Bmp4* during the early stages of UB outgrowth (Hsu et al., 1998; Michos et al., 2007; Michos et al., 2004). *Grem1*-deficient embryos show arrested UB outgrowth and the majority of animals are born without metanephric kidneys. These defects are rescued following inactivation of one copy of the *Bmp4* gene, consistent with the *Grem1* null phenotype being caused by excessive BMP4 signaling (Michos et al., 2007; Michos et al., 2004). Expression of *Gdnf* in the *Grem1* mutant metanephric mesenchyme is initially normal but is downregulated progressively and lost by E11.75. While these observations suggest that locally inhibiting BMP4 signaling around the nascent UB is needed to maintain *Gdnf* expression, treatment of isolated metanephric mesenchyme with either BMP4 or *Grem1* does not alter *Gdnf* expression. An elevated number of cells positive for phosphorylated Smad1/5/8 proteins, which mediate canonical BMP signaling, were found in the metanephric mesenchyme of *Grem1* mutants (Michos et al., 2007). However, the importance of this is unclear as inactivation of *Smad4*, encoding the common Smad co-factor, in the metanephric mesenchyme does not affect UB outgrowth (Oxburgh et al., 2004). Thus, the mechanism-of-action by which BMP4 inhibits UB outgrowth and whether the nephric duct or metanephric mesenchyme is the target tissue that responds to BMP4 remain unclear.

### **Survival, proliferation, and condensation of the metanephric mesenchyme**

The UB invades the metanephric blastema at E11 and induces the condensation of a subset of metanephric mesenchyme cells around the duct tip. These cells, known as ‘induced mesenchyme’ or ‘cap mesenchyme’, form a layer 4–5 cells thick that are morphologically distinguishable from the more peripheral ‘uninduced’ mesenchyme. Cap mesenchyme cells express a range of transcription factors, including *Pax2*, *Eya1*, *Six2*, *Wt1*, *Sal1*, many of which are initially activated in the metanephric mesenchyme, as well as secreted molecules such as GDNF and BMP7 (see Figure 5). The cap mesenchyme is essential for inducing UB branching as well as providing a source of progenitor cells that differentiate into nephrons (see below). Therefore, the formation and preservation of these cells is critical for metanephros formation.

At least three signals are needed to induce and maintain the cap mesenchyme. First, a survival signal must prevent the metanephric mesenchyme from executing a default apoptotic pathway (Coles et al., 1993; Grobstein, 1955; Koseki, 1993; Weller et al., 1991). Failure to induce survival of the metanephric mesenchyme results in programmed cell death and kidney agenesis. This phenotype is seen in mutants that fail to form a UB outgrowth or when the metanephric mesenchyme is physically separated from the UB *in vitro* (Koseki et al., 1992). Second, the cap mesenchyme must be induced to proliferate in order to ensure that new UB tips acquire a sufficient number of cells to maintain subsequent rounds of branching and nephron formation. In this regard, the cap mesenchyme is considered to represent a pool of self-renewing progenitor cells. A recent fate mapping study has shown that *Six2*-expressing cap mesenchyme cells display a tremendous capacity for expansion and are able to increase their number by 15-fold in 8 days (Kobayashi et al., 2008). Third, a signal promotes the condensation/recruitment of metanephric mesenchyme around the UB tip. This signal is poorly understood but appears to depend on *Smad4*, encoding an intracellular mediator of TGF- $\beta$ /BMP signaling, as conditional inactivation of *Smad4* in the metanephric mesenchyme results in the cap mesenchyme failing to efficiently coalesce around the

UB tips (Oxburgh et al., 2004). The transcriptional regulation of survival, anti-apoptotic, and condensation factors in the UB is not well understood but may be downstream of the *Emx2* homeobox transcription factor that is expressed by the UB. *Emx2* mutant embryos show normal UB outgrowth but the metanephric mesenchyme fails to proliferate or condense around the UB tips followed by the degeneration of the metanephric mesenchyme and UB by E13 (Miyamoto et al., 1997).

Members of the Wnt, FGF, and BMP families of secreted factors, as well as Pax2 and Pax8, have been implicated as anti-apoptotic and/or pro-growth factors for the metanephric mesenchyme, and likely act in a partially redundant fashion (Barasch et al., 1997; Dudley et al., 1999; Dudley and Robertson, 1997; Grieshammer et al., 2005; Luo et al., 1995; Narlis et al., 2007; Oxburgh et al., 2005; Perantoni et al., 1995; Poladia et al., 2006; Schmidt-Ott et al., 2007). There is a clear *in vivo* requirement for BMP7 to function as an anti-apoptotic factor for the metanephric mesenchyme. *Bmp7* is expressed in the UB and cap mesenchyme and while the initial stages of UB outgrowth and branching occur normally in *Bmp7* mutants, the metanephric mesenchyme starts undergoing apoptosis at E13.5 and is lost by E16.5 (Dudley et al., 1999; Dudley and Robertson, 1997; Luo et al., 1995; Lyons et al., 1995). As a result, *Bmp7* deficient embryos exhibit a depletion of the cap mesenchyme, premature termination of kidney development, and reduced UB branching. Explant experiments further support a role for BMP7 as a survival factor for the cap mesenchyme and also suggest that it may help maintain these cells in an undifferentiated state (Dudley et al., 1999). These effects are probably shared by other *Bmps* expressed in the kidney, such as *Bmp2* and *Bmp4*, as a gene 'knock-in' approach demonstrated that *Bmp4* could fully substitute for *Bmp7* (Oxburgh et al., 2005).

### **Molecular regulation of ureteric bud branching** **Glial-derived neurotrophic factor (GDNF)**

In addition to promoting UB outgrowth, GDNF-*Ret* signaling also plays a central role in subsequent UB branching, probably by stimulating the cell migration and proliferation that characterizes the UB tip (Costantini and Shakya, 2006). Following UB invasion of the metanephric mesenchyme, *Gdnf* becomes expressed in the cap mesenchyme surrounding each UB tip (Hellmich et al., 1996; Sainio et al., 1997). This restricted expression pattern is not essential for GDNF function, as transgenic expression of *Gdnf* throughout the ureteric epithelium of *Gdnf*-null mutants does not significantly alter UB branching (Shakya et al., 2005). Expression of *Ret* becomes localized to the UB tips and maintenance of this expression pattern is dependent upon retinoic acid signaling, consistent with earlier observations linking vitamin A/retinol deficiency with renal malformations (Batourina et al., 2001; Mendelsohn et al., 1999; Wilson and Warkany, 1948). Based on the expression of retinoic acid receptors, retinoic acid is not thought to signal directly to the UB but is instead hypothesized to stimulate the release of an unknown *Ret*-inducing factor from surrounding stromal cells (Batourina et al., 2001; Dolle et al., 1990; Mendelsohn et al., 1999). Whether this factor stimulates *Ret* expression in the UB or acts via the metanephric mesenchyme is not known. The localization of *Ret* to the UB tip is important for branching morphogenesis as misexpression of *c-ret* throughout the ureteric epithelia has been shown to inhibit UB growth and branching in transgenic mice, possibly by acting as a 'sink' for GDNF (Srinivas et al., 1999).

*Ret* is a downstream target of GDNF signaling and forms part of a positive feedback loop (see Figure 5). Another component of this auto-regulatory loop is *Wnt11*, which is dependent upon GDNF signaling for its UB tip-specific expression pattern and which contributes to the

maintenance of *Gdnf* expression in the adjacent cap mesenchyme (Kispert et al., 1996; Majumdar et al., 2003; Pepicelli et al., 1997). However, the branching defect in *Wnt11* mutant embryos is relatively mild, most likely because *Gdnf* expression levels are only slightly reduced. Expression of *Wnt11* is downregulated in the UB tips of *Pax2/Pax8* compound heterozygotes, which also show a reduced UB branching phenotype, suggesting that the stimulatory effects of GDNF on *Wnt11* may be mediated via the *Pax2/8* transcription factors (Narlis et al., 2007). *Wnt11* can signal via the non-canonical planar cell polarity pathway, which has been implicated as a modulator of cell shape and migration, therefore, in addition to participating in a GDNF positive feedback loop, *Wnt11* may also act in a pathway parallel to the GDNF-Ret axis (Tada et al., 2002).

Sprouty1 also plays a role in regulating UB branching in response to GDNF-Ret signaling. *Spry1* is expressed in the ureteric tree and at a much lower level in the cap mesenchyme. In conventional *Spry1* mutants and in conditional mutants lacking *Spry1* in the UB-derived epithelium, the UB displays increased budding/branching with significantly larger ureteric trunks and dilated UB tips (Basson et al., 2006). These defects were associated with ectopic expression of *Wnt11* in the connecting segments and increased expression of *Gdnf* in the cap mesenchyme. These results are consistent with the ureteric epithelium in *Spry1* mutants being hypersensitive to GDNF, leading to ectopic *Wnt11* expression, and a corresponding upregulation of *Gdnf* in the mesenchyme. The increased level of GDNF-Ret signaling at the UB tip is then hypothesized to be the primary cause of the observed branching defects in *Spry1* mutants.

## Wnts

Wnt signaling via the canonical  $\beta$ -catenin pathway has been implicated in UB branching (Bridgewater et al., 2008; Maretto et al., 2003; Moriyama et al., 2007). Studies with transgenic reporter mice have demonstrated that  $\beta$ -catenin-induced genes are active in the UB during branching morphogenesis (Maretto et al., 2003; Moriyama et al., 2007). UB-specific inactivation of  $\beta$ -catenin arrests branching at E12.5 resulting in renal aplasia or renal dysplasia (Bridgewater et al., 2008). In addition, this phenotype is associated with reduced expression of *Gdnf* and *Ret* in the cap mesenchyme and UB tip, respectively. A number of Wnts capable of signaling through the canonical  $\beta$ -catenin pathway are expressed in the developing metanephros, including *Wnt6*, *Wnt7b*, and *Wnt9b* in the collecting duct system, and *Wnt4* in early nephron precursors (Merkel et al., 2007). Of these, loss of *Wnt4* or *Wnt9b* leads to a disruption in UB branching after the T-stage, with the *Wnt9b* mutant phenotype being more severe than the *Wnt4* mutant phenotype (Carroll et al., 2005; Stark et al., 1994). The observation that a branching defect occurs following loss of *Wnt4*, which is expressed in the renal vesicle below the UB tip, suggests a feedback mechanism in which nephrons induced in response to branching use *Wnt4* to promote further UB branching (see Figure 5). In *Wnt9b* mutant embryos, expression of *Wnt11* and *Gdnf* is downregulated prior to the morphological appearance of the branching defect (Carroll et al., 2005). However, given the reciprocal nature of interactions occurring between the cap mesenchyme and the UB tip and the existence of a GDNF-Ret auto-regulatory loop, it is not known if the cellular target of *Wnt9b* activity is the cap mesenchyme or the UB.

The identities of the genes regulated by Wnt signaling in the UB are still being elucidated. An analysis of kidneys deficient in  $\beta$ -catenin in the UB has identified *Emx2* as a possible early Wnt target (Bridgewater et al., 2008). In addition to the metanephric mesenchyme survival phenotype mentioned above, *Emx2* mutants exhibit decreased expression of *Lhx1* and *Ret* in the UB (Miyamoto et al., 1997). UB-specific deletion of *Lhx1* also causes reduced *Ret* expression and

reduced UB branching, leading to the hypothesis that a  $\beta$ -catenin-*Emx2*-*Lhx1*-*Ret* pathway may be involved in maintaining *Ret* expression during UB branching (Bridgewater et al., 2008; Kobayashi et al., 2005).

### **Fibroblast growth factors**

*In vitro* studies have demonstrated that transient addition of multiple FGFs to kidney organ cultures increases UB growth and branching (Qiao et al., 2001; Qiao et al., 1999). Corroborating *in vivo* evidence that FGFs participate in UB branching comes from the genetic manipulation of *Fgfr2*. UB-specific deletion of *Fgfr2* results in small metanephroi with long, thin ureteric trunks and fewer UB tips (Zhao et al., 2004). Similar, but less severe, growth and branching phenotypes are seen in mutants singly deficient in *Fgf7*, *Fgf10*, and *Fgfr2IIIb* (the main receptor isoform for FGF7 and FGF10 (Ohuchi et al., 2000; Qiao et al., 1999; Revest et al., 2001)).

### **Bone Morphogenetic Proteins**

BMP signaling plays a role in UB branching. Transcripts for the closely related genes *Bmp2* and *Bmp4* are found in the mesenchyme surrounding the tips of the branching UB and stromal cells surrounding the nephric duct and the stalk of the UB outgrowth (future ureter), respectively (Dudley and Robertson, 1997). Organ culture experiments have shown that BMP4 can inhibit UB branching directly and at least part of this effect is due to an antagonism of GDNF signaling (Brophy et al., 2001; Bush et al., 2004; Cain and Bertram, 2006; Martinez et al., 2002; Miyazaki et al., 2000; Raatikainen-Ahokas et al., 2000). UB-specific expression of a constitutively active *Alk3* transgene, encoding the BMP receptor type IA, results in decreased branching (Hu et al., 2003). UB-specific loss of *Alk3* causes a biphasic branching defect that is characterized by an early increase in branching followed by a decrease that results in an overall reduction in UB tip number (Hartwig et al., 2008). These findings suggest that ALK3 signaling normally functions to limit UB branching during early stages of branching morphogenesis. The later branching defect caused by *Alk3* deficiency is postulated to be secondary to the early abnormal branching pattern. No alterations in the rates of cell proliferation or apoptosis were found in the *Alk3* mutants and nor were there any qualitative differences in the expression levels of *Gdnf*, *Ret*, *Wnt11* at E11.5 compared to wild-type kidneys (Hartwig et al., 2008). Thus, the downstream targets mediating the effects of *Alk3* signaling on UB branching are unclear. Surprisingly, UB-specific deletion of *Smad4* has no effect on UB branching, indicating that BMPs must exert their effects via a *Smad4*-independent pathway (Oxburgh et al., 2004).

### **Stromal cells**

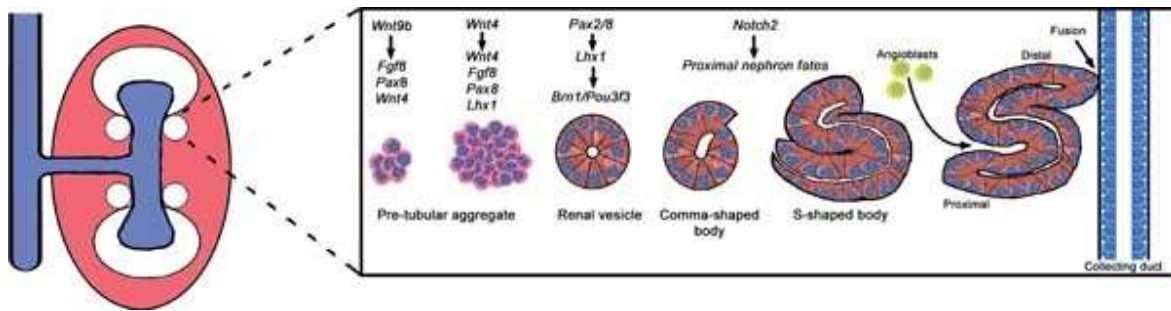
In addition to the UB and the cap mesenchyme, renal stromal cells have been identified as an important source of metanephric regulatory signals, such as the production of retinoic acid as described above. Traditionally, these cells been assumed to arise from the uninduced metanephric mesenchyme but more recent evidence suggests that they may derive from cells surrounding the metanephric mesenchyme (Cullen-McEwen et al., 2005). By E14, stromal cells can be seen in three major layers: the capsular stroma, consisting of a single continuous layer of flattened cells at the outside edge of the kidney, cortical stroma, surrounding the cap mesenchyme and UB tips towards the periphery, and the medullary stroma (Cullen-McEwen et al., 2005).

Cortical and capsular stromal cells express the *Foxd1* transcription factor and inactivation of this gene reduces UB branching and causes disorganized and delayed nephron formation (Hatini et al., 1996; Levinson et al., 2005). Associated with this phenotype is an expansion of the cap mesenchyme, suggesting that the stroma plays an important role in controlling the rate of cap mesenchyme differentiation and/or self-renewal. A more recent analysis of *Foxd1* mutants has revealed that the maturation of the kidney capsule is abnormal and instead of forming a single homogenous layer of cells, a thicker layer that fails to express capsule markers is formed. Included in the mutant capsule are ectopic *Bmp4*-expressing cells that are hypothesized to inappropriately signal to the cap mesenchyme and UB tips leading to delayed and disorganized nephron formation and reduced UB branching (Levinson et al., 2005). These findings highlight the importance of the stromal cells in establishing a signaling environment that is conducive to normal metanephric growth and development.

Cap mesenchyme and branching defects similar to that seen in *Foxd1* mutants are also seen in embryos defective in the *Pbx1* and *Pod1* transcription factors, however the epistatic relationships between these genes and *Foxd1* is not clear (Quaggin et al., 1999; Schnabel et al., 2003).

### **Metanephric nephron development**

Nephron formation begins at E11.5 with the appearance of small clusters of mesenchymal cells on either side of the unbranched UB tip. These pre-tubular aggregates initially consist of 4–6 cells that are contiguous with the cap mesenchyme but undergo rapid proliferation to form a cluster of approximately 30 cells (Bard et al., 2001). Lineage labeling experiments both *in vitro* and *in vivo* have confirmed that the cap mesenchyme gives rise to all of the epithelial segments of the nephron (podocytes through to the distal tubule; Boyle et al., 2008; Herzlinger et al., 1992; Kobayashi et al., 2008; Osafune et al., 2006). Following UB branching and growth, the pre-tubular aggregates become located beneath the UB tips (known as the proximal side). Similar to the morphogenesis seen for mesonephric nephrons, the pre-tubular aggregates undergo a mesenchymal-to-epithelial transition to form renal vesicles at E12.5 and then proliferate to give rise to comma- and S-shaped bodies that fuse with the collecting duct epithelium (see Figure 6).



**Fig. 6: Metanephric nephron formation.**

Nephrons arise from pretubular aggregates, a subpopulation of the cap mesenchyme, in response to Wnt9b. Early molecular markers of pretubular aggregates are *Pax8*, *Fgf8*, and *Wnt4*. Wnt4 maintains the expression of these genes, induces *Lhx1*, and converts the pretubular aggregate into an epithelial vesicle. The renal vesicle displays polarized expression of the *Brn1/Pou3f3* transcription factor gene in presumptive distal nephron precursors. Growth of the renal vesicle leads to the stereotypical formation of comma- and S-shaped bodies. Notch2 signaling is necessary for the specification of proximal nephron fates (podocytes and proximal tubule). Angioblasts invade the proximal cleft of the S-shaped body and contribute to the glomerular capillary. The distal portion of the S-shaped body fuses with the collecting duct.

Based on restricted gene expression patterns, the S-shaped body can be subdivided into podocyte progenitors and the precursors of the proximal and distal tubule segments, although definitive lineage labeling experiments have not yet been done (Dressler, 2006). The cells that make up the cleft at the proximal end of the S-shaped body (farthest away from the collecting duct) express *Wt1* and mature into podocytes. These cells also produce VEGF and attract angioblasts that contribute to the formation of the glomerulus (Eremina et al., 2003). As proliferation of the nascent nephron continues, the proximal and distal tubule segments become convoluted while the region between them grows down into the medullary zone to form the Loop of Henle (Neiss, 1982). Nephrons located in superficial or midcortical regions of the kidney possess short loops of Henle that turn within the cortex or outer medulla zone. More deeply located nephrons (juxtamedullary nephrons) have long loops of Henle that descend into the inner medulla region (Hebert et al., 2001). In addition to the loop of Henle, metanephric nephrons are also distinguished from other nephron types by the macula densa, a population of salt- and fluid-sensing cells in the lining of the distal tubule (see Figure 1). These cells make contact with the parent glomerulus of the nephron and form part of a tubuloglomerular feedback system that couples distal nephron flow with glomerular filtration rate and ensures that the flow through the nephron is tightly controlled (Castrop, 2007).

### Molecular mechanisms of metanephric nephron formation

Gene targeting experiments identified Wnt9b and Wnt4 as being necessary for pre-tubular aggregate formation and renal vesicle formation, respectively (Carroll et al., 2005; Kispert et al., 1998; Stark et al., 1994). *Wnt9b* is expressed throughout the UB-derived collecting duct system (excluding the UB tips) and is considered the primary UB-derived paracrine signal that induces the formation of pre-tubular aggregate cells from the cap mesenchyme (Carroll et al., 2005). Despite the widespread expression pattern of *Wnt9b* throughout the collecting system only a small subset of cap mesenchyme cells activate a nephrogenic program, thus indicating that additional regulatory molecules must limit the action of Wnt9b. One such factor is *Six2*, which is expressed



by the cap mesenchyme and functions to suppress the ectopic formation of pre-tubular aggregates on the distal side of the UB tip (Kobayashi et al., 2008; Self et al., 2006). Ectopic nephron induction does not occur in compound mutants defective in both *Six2* and *Wnt9b*, consistent with *Six2* acting as a suppressor of the nephron-inducing activity of *Wnt9b* (Kobayashi et al., 2008). However, the nature of the *Six2*-mediated repression of nephrogenesis and whether it acts directly on Wnt signaling is not known.

*Wnt4* is expressed by the pre-tubular aggregates and is postulated to work in an autocrine manner to propagate the initial Wnt signal and complete the transition to the renal vesicle stage. Both *Wnt9b* and *Wnt4* signal via the canonical  $\beta$ -catenin pathway but may use different receptors and act in a linear pathway as *Wnt9b* is unable to induce nephrogenesis in *Wnt4* mutant mesenchyme *in vitro* (Park et al., 2007). Gain-of-function studies examining the effect of sustained  $\beta$ -catenin signaling in the metanephric mesenchyme suggest that although Wnt signaling is essential during the early phases of the nephrogenic program, it must be attenuated at later stages in order for nephron progenitors to undergo a mesenchymal-to-epithelial transition (Kuure et al., 2007; Park et al., 2007; Schmidt-Ott et al., 2007).

A number of studies have begun unraveling the epistatic interactions between Wnt signaling and functionally important nephrogenic genes. Although the circuitry is likely to be more complex, current data suggest that *Wnt9b* initially activates the expression of *Wnt4*, *Fgf8*, and *Pax8* in the pre-tubular aggregate, and then *Wnt4* maintains the expression of these genes and induces *Lhx1* (perhaps in conjunction with FGF8; Grieshammer et al., 2005; Kobayashi et al., 2005; Stark et al., 1994). *Lhx1* transcripts are significantly downregulated in *Pax2/Pax8* compound heterozygotes (consistent with similar observations found in the nephric duct; see section 4), whereas expression of *Wnt4* and *Fgf8* are unaffected (Narlis et al., 2007). These data suggest that *Pax2* and *Pax8* may act downstream of *Wnt4* to induce/maintain *Lhx1* expression in the pre-tubular aggregate and renal vesicle. In addition, *Pax2* and *Pax8* are required for nephron survival (Narlis et al., 2007). Conditional inactivation of *Lhx1* in the cap mesenchyme has demonstrated a requirement of *Lhx1* for the progression of the renal vesicle to the comma-shaped body stage (Kobayashi et al., 2005; Potter et al., 2007). Interestingly, expression of the secreted Wnt antagonist *Dkk1* is down-regulated in the renal vesicles of *Lhx1* mutants raising the possibility that a *Wnt4-Pax2/8-Lhx-Dkk1* pathway may provide the negative feedback loop needed to attenuate Wnt signaling following the initial activation of the nephrogenic program (Potter et al., 2007).

Little is known about the mechanisms responsible for generating the segmentation pattern of the nephron. Current data suggest that proximo-distal patterning begins very early during nephrogenesis as transcripts for *Brn1/Pou3f3*, encoding a POU domain transcription factor required for distal tubule formation, are restricted to a subset of renal vesicle cells closest to the UB epithelium (Nakai et al., 2003). This localized expression domain is absent in conditional *Lhx1* mutants, leading to the suggestion that *Lhx1* may act upstream of *Brn1/Pou3f3* to establish an early proximo-distal pattern in the renal vesicle (Kobayashi et al., 2005). Interestingly, *Pax2/Pax8* compound heterozygotes also display a loss or reduction of the distal tubule segment, consistent with *Pax2/8* acting upstream of *Lhx1* (Narlis et al., 2007). Both *Pax2/8* and *Lhx1* are initially expressed throughout the renal vesicle therefore it is not clear how expression of *Brn1/Pou3f3* is restricted to presumptive distal progenitors. From early observations, it was hypothesized that the proximo-distal patterning of the nascent nephron occurred in response to signals emanating from the UB (Saxén, 1987). Whether either a *Wnt4* or *Wnt9b* gradient provides such a signal remains to be determined.

There is good evidence to indicate that the Notch signaling pathway is involved in proximo-distal patterning where it appears to act between the renal vesicle and S-shaped body stages to promote the formation of podocytes, proximal tubules, and the loop of Henle (Cheng et al., 2007; Cheng et al., 2003; Wang et al., 2003). At present the downstream transcriptional targets of Notch in the metanephric nephron are not known. Work in *Xenopus* suggests that one of the members of the Hey family of transcription factors, which are known targets of Notch, may mediate the effects of Notch signaling in the pronephros (Taelman et al., 2006). However, a role for Hey orthologues in metanephric nephron patterning has yet to be demonstrated. In addition to Notch, the RA pathway has been implicated in proximo-distal nephron patterning of the zebrafish pronephric nephron (Wingert et al., 2007). Whether RA is involved in metanephric nephron patterning, in addition to its effects on UB branching, is unknown.

### **Concluding remarks**

Kidney development in amniotes is unique with respect to the other organs in that three separate kidney types arise during embryogenesis. From an evolutionary perspective, the acquisition of additional kidney structures is thought to have occurred during the transition of a freshwater protovertebrate to a dry terrestrial environment (Smith, 1959). The pronephric kidney in lower vertebrates with freshwater larvae, such as *Xenopus* and zebrafish, plays a critical role in water excretion and prevents the embryo from dying an early death by edema. As the animal grows, additional renal function is necessary in order to cope with the added demands on waste excretion and fluid homeostasis. Therefore, the mesonephric kidney may have evolved in response to greater body mass and associated factors such as increased blood pressure (Vize et al., 1997). The metanephric kidney, with its unique water conserving ability, was the adaptation that allowed amniotes to live and breed on dry land.

In mammals, the embryo is protected from osmotic challenges coming from the external environment by virtue of *in utero* development, while the problem of waste excretion is ameliorated by the allantois and placenta. Thus, the mammalian embryo has a reduced requirement for the pronephric and mesonephric kidneys and in the case of the mouse these have regressed to rudimentary structures that are short-lived. The (pro)nephric duct has been retained, as the metanephric kidney is dependent upon it as the source of the ureteric epithelium, while some of the mesonephric tubules are co-opted in males for the epididymal ducts of the testis.

Each kidney type is comprised of similar cell types and performs common renal functions. It is not surprising then, that the genes involved in the formation of one kidney type are re-employed in the others. This means that the study of gene function in one kidney type, such as the use of *Xenopus* and Zebrafish to examine genetic hierarchies involved in pronephros development, is likely to yield information that is useful to all kidney types. Ultimately however, a detailed understanding of metanephric kidney development can only come from the study of these genes in mammals. In the past, analyzing the function of murine genes central to the development of all kidney types was hampered by early nephric duct defects preventing an assessment of gene function in the metanephros. This has now been largely overcome by employing Cre-Lox technology and the generation of nephric duct- and metanephric mesenchyme-restricted Cre recombinase lines. As additional lines are made, it should be possible to inactivate genes in smaller, lineage-restricted populations of cells, thus providing the level of precision and temporal control needed to unravel the complex epistatic relationships currently hindering the field. A number of renal genes have been implicated in human disease, including *PAX2* (renal-coloboma syndrome),

*WT1* (Wilms' tumor and urogenital disorders), *SIX1* and *EYAI* (branchio-oto-renal syndrome), *GATA3* (HDR syndrome), and *SALL1* (Townes-Brocks syndrome; (Dressler, 2006). Thus, a greater understanding of their role during metanephric kidney development will provide a molecular basis for the pathogenesis of these diseases and may lead to new therapeutic or preventative treatments.

Knowledge of embryonic kidney development will also form the foundation for developing stem cell-based strategies to treat renal disease. Some of these approaches are based on the directed differentiation of embryonic stem (ES) or other multipotent cells into kidney cells using cocktails of growth factors followed by transplantation into embryonic or adult kidneys (Bruce et al., 2007; Kim and Dressler, 2005; Kobayashi et al., 2005; Schuldiner et al., 2000; Vigneau et al., 2007). At present, this approach has yielded limited success but will likely improve with a better understanding of the genetic hierarchies governing the formation of the different kidney types. For example, in order to make metanephric mesenchyme from pluripotent stem cells it may be necessary to first convert these cells into *Pax2/8*<sup>+</sup> and *Odd1*<sup>+</sup> intermediate mesoderm and then direct them into a metanephric mesenchyme fate by inducing factors such *Eya1*, *Six2*, and *Hox11*. Recent studies *in vivo* have shown that ectopic expression of *Hox11* in cranial portions of the nephrogenic cord, where it is not normally expressed, is sufficient to induce *Six2* expression and transform mesonephric nephrons into more metanephric-like nephrons (Mugford et al., 2008). Thus, the activation of single regulatory factors in stem cell-derived intermediate mesoderm may be sufficient to initiate metanephric mesenchyme formation *in vitro*. It is unreasonable to hope for the generation of a complete kidney *in vitro*, however specific cell types, such as the cap mesenchyme, may have therapeutic potential. In the mouse, the cap mesenchyme is lost at postnatal day 3 and nephrogenesis ceases abruptly. In contrast, the UB still expresses *Wnt9b* at this time and retains the ability to induce nephrons and promote embryonic metanephric mesenchyme survival *in vitro* (Hartman et al., 2007). Although it is not known how long the nephron-inducing activity of the postnatal UB lasts, it raises the possibility that the transplantation of ES-cell derived cap mesenchyme into newborn or adult kidneys could lead to a restoration of nephrogenesis and a potential therapy for renal defects and disease.

The ability to differentiate pluripotent stem cells into renal cells *in vitro* will also be useful for studying kidney disease in humans. The recent discovery that somatic cells can be converted into ES-like cells, called induced pluripotent stem cells (iPS) now makes it relatively easy to create a multitude of disease-specific iPS lines (Park et al., 2008; Yamanaka, 2008). Being able to create specific renal cell lineages, such as proximal and distal tubular epithelial cells and podocytes, from patients with renal disorders will provide valuable *in vitro* tools to dissect the pathogenesis of diseases such as glomerulopathies and polycystic kidney disease.

One of the limitations of using pluripotent stem cell-derived tissues for cell-based therapies is the risk of teratoma formation (Little, 2006). Hence, there is an ongoing search for a resident renal stem cell that would comprise a more therapeutically tractable population to target. The mammalian kidney is known to possess a limited ability to repair the tubular epithelium of damaged nephrons (Humphreys and Bonventre, 2007). Whether this repair is mediated by the dedifferentiation and proliferation of surviving epithelial cells or whether a resident renal stem cell is involved has become the subject of recent investigation. Although candidate renal stem cells have been found in the renal papilla, tubular epithelium, and the urinary pole of the glomerulus, *in vivo* genetic lineage-labeling and serial transplantation experiments are needed in order to confirm their stem cell properties (Gupta et al., 2006; Lazzeri et al., 2007; Maeshima, 2007; Maeshima et al., 2006; Oliver et al., 2004; Sagrinati et al., 2006). A recent fate-mapping analysis of ischemia-

reperfusion injured kidneys showed that surviving tubular epithelial cells are the predominant source of the regenerated tissue (Humphreys et al., 2008). While this experiment rules out an extra-tubular source of renal stem cells, it does not exclude the possibility that a resident epithelial progenitor/stem cell is responsible for the repair. Alternatively, each epithelial cell in the nephron may possess an inherent ability to dedifferentiate, proliferate, and re-differentiate into an epithelial cell following damage. There is some evidence to suggest that re-differentiating epithelial cells express embryonic renal genes such as *Pax2* (Villanueva et al., 2006). This suggests that tubular regeneration may recapitulate aspects of renal development. If so, then the study of embryonic kidney development may provide insights into the mechanism of renal repair following acute kidney injury.

In summary, elucidating the molecular basis of kidney development will increase our understanding of renal birth defects and disease, provide a foundation for directing the differentiation of pluripotent stem cells into clinically useful renal cells, and further our knowledge of epithelial repair following kidney injury. While there is still much to be learnt in each of these arenas, there is clearly much to be gained.

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