Advances in early kidney specification, development and patterning

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The kidney is a model developmental system for understanding mesodermal patterning and organogenesis, a process that requires regional specification along multiple body axes, the proliferation and differentiation of progenitor cells, and integration with other tissues. Recent progress in the field has highlighted the essential roles of intrinsic nuclear factors and secreted signaling molecules in specifying renal epithelial stem cells and their self-renewal, in driving the complex dynamics of epithelial cell branching morphogenesis, and in nephron patterning. How these developments influence and advance our understanding of kidney development is discussed.

Introduction

The study of development concerns itself with some of the most daunting and conceptually difficult problems in biology. How do specialized cells and tissues differentiate from their more simple progenitors during embryonic development and become organized into a three-dimensional architectural framework? At what point along a lineage pathway are cells committed to a particular fate and how is that fate remembered over many cell divisions? What enables self-renewal in a stem cell and how does it decide whether to 'spin-off' more differentiated progenitors? How are developmental regulatory pathways recruited in the initiation and progression of human disease states? All of these important and interrelated questions lend themselves to investigation in many model organisms and tissues. In this article, I will summarize recent progress in the area of kidney development that touches on all of these issues. As such, I would also like to stress how well the kidney has served as a model organ for developmental biologists whose interests span the gamut from patterning to cellular differentiation and human disease.

As a group, developmental biologists have been rather opportunistic and clever in their choice of model organisms. From amphibians to fruit flies and nematodes, there seemed to be just the right organism for any problem. Yet ultimately, we would like to know something about ourselves and how we humans are formed. Couple this desire with the priorities of many of our funding institutions, which are to promote health and well being, and it becomes increasingly clear that we must study mammalian tissues and organs, not just as they develop, but as they age and as they respond to environmental insults. Ideally, we must be able to utilize what we learn in model systems and to translate this to mammalian organisms, just as we ought to be able to test hypotheses that may have originated from mammalian studies by transposing them into simpler organisms. The mammalian kidney has proved to be just such a model tissue. Since the pioneering work of Grobstein (Grobstein, 1956), the kidney has been studied in mammals, frogs, fish and chick, and even the *Drosophila* Malphigian tubules and trachea have utility as model systems for epithelial cell polarity,

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branching morphogenesis and patterning. More recently, progress in understanding the specification and organogenesis of the kidney has had significant impact, not only for understanding basic development and patterning of the mesoderm, but also for stem cell renewal, segmentation and boundary formation, and signaling pathways. In this article, I discuss some of the more significant recent advances and concepts that have emerged from the kidney development field, how they might be relevant to the general developmental community, and the key outstanding questions that they raise.

Early regionalization

In order to fully appreciate the recent advances made in deciphering the molecular events that pattern the kidney field, it is important to recognize the temporal and spatial organization of the embryonic kidney and its progenitors. After gastrulation in mammals, the kidney develops from the intermediate mesoderm as a continuum along the anteroposterior axis in a distinct temporal sequence (Fig. 1) (Dressler, 2006; Saxen, 1987). Anterior kidney structures include the pro- and mesonephros, whose complexity, size and duration vary greatly among vertebrate species. In the mouse, the pronephros is barely detectable, whereas mesonephric tubules are well developed with a proximal glomerulus and convoluted tubules that empty into the nephric duct (Fig. 1B-E). The adult, or metanephric kidney, forms at the posterior end of this intermediate mesoderm. Thus, the intermediate mesoderm (IM) must require both mediolateral patterning and anteroposterior patterning signals to determine the kidney field.

The origin of the IM has been explored in a number of experimental organisms. Fate mapping in the mouse gastrula has demonstrated that paraxial mesoderm is primarily derived from the more anterior primitive streak, whereas lateral plate mesoderm (LPM) is derived from cells migrating through the more posterior primitive streak (Fig. 2A,B) (Kinder et al., 1999; Kinder et al., 2001; Parameswaran and Tam, 1995). Defining the IM at the early post-gastrula stage is not easy, as very few molecular markers are specific just for this region (Fig. 2C). Expression of the LIM-type homeobox gene *Lhx1* is evident in the prospective LPM at the late-streak stage, and is one of the first markers for this posterior, lateral mesoderm (Tsang et al., 2000). More recently, the odd-skipped related gene *Osr1*, which encodes a zinc-finger DNA-binding protein, was identified as another early marker for the more LPM in chick and mouse (James et al., 2006; James and Schultheiss, 2005). Expression domains for *Osr1* and *Lhx1* overlap and encompass the prospective IM as well as the more LPM, with *Osr1* being expressed along the entire AP axis from the first somites (Fig. 2C). It is not until about the 4- to 8-somite stage that markers more exclusive to the IM are observed. The *Pax2* and *Pax8* genes are activated within the IM from approximately the 6th somite, in a very narrow stripe of cells just lateral to the paraxial mesoderm, but their expression does not extend into the more lateral plate (Bouchard et al., 2002). Shortly thereafter, *Lhx1* expression becomes more restricted to the IM and
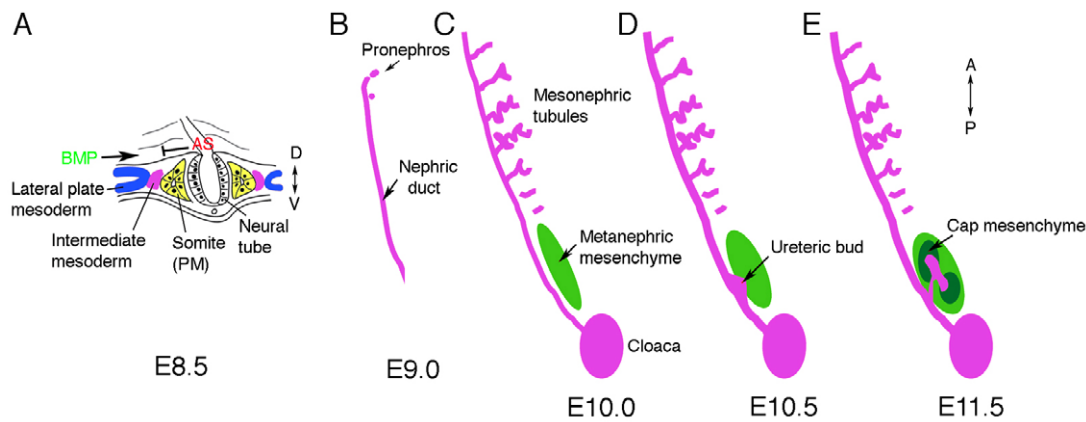
Fig. 1. The intermediate mesoderm: its origin and derivatives. In amniotes, the kidney arises from the intermediate mesoderm (IM), between the paraxial somatic mesoderm (PM) and the lateral plate mesoderm (LPM). (**A**) Schematic cross section through a mouse embryo at embryonic day 8.5 (E8.5) at approximately the sixth somite. The presumptive IM (purple) forms between the LPM (blue) and the PM (yellow). Antagonistic signals (AS) from somites might counteract BMP signals from the LPM to generate the pronephric field. (**B-E**) Schematics of the IM-derived kidney structures that develop along the anteroposterior (AP) axis in a specific temporal and spatial order. (B) The Wolffian, or pronephric, duct is visible at E9.0 in the mouse and grows caudally by proliferation and extension, inducing epithelial tubules from the adjacent mesenchyme. The pronephros is very rudimentary. (C) Mesonephric tubules at E10, as the nephric duct reaches the cloaca, are more developed in the mid-thoracic region, with a vascularized glomerulus at the proximal end and convoluted tubules draining into the nephric duct. Posterior cells adjacent to the duct form an aggregate called the metanephric mesenchyme (green). (D) By E10.5, an outgrowth of the duct, the ureteric bud (UB), invades the metanephric mesenchyme. (E) By E11.5, the UB has bifurcated and induced mesenchyme (cap mesenchyme) surrounds the tips. Cap mesenchymal cells are the epithelial stem cells of the nephron and generate the glomerular podocyte cells, the parietal epithelium, the proximal tubules, and the distal tubules.

to the nephric duct as it begins to form and extend caudally. *Osr1* remains expressed in the mesenchymal cells surrounding the nephric duct and in the more LPM derivatives, but is excluded from the Pax2-positive cells of the nephric duct itself.

Genetic analyses in mice point to *Lhx1*, *Osr1* and *Pax2/Pax8* having critically important roles in early specification of the IM, yet their epistatic relationships remain unclear. Phenotypically, mice homozygous for an *Lhx1* null mutation show no morphological evidence of nephric duct formation, although *Pax2* expression is observed in cells at the boundary between the paraxial and lateral plate mesoderm shortly after gastrulation (Tsang et al., 2000). The *Pax2* null mutants do develop a nephric duct (Brophy et al., 2001; Torres et al., 1995), but the duct is completely absent in a *Pax2;Pax8* double mutant, suggesting that these Pax genes function redundantly in this early IM domain (Bouchard et al., 2002). The *Pax2;Pax8* double mutants also do not express *Lhx1*. Oddly enough, mice homozygous for an *Osr1* null allele, the expression of which precedes that of *Pax2* and *Pax8*, still exhibit nephric duct formation and *Pax2* expression in the anterior IM, yet they lack more developed mesonephric tubules and the metanephric mesenchyme in the posterior IM (James et al., 2006; Wang et al., 2005). Whether this anterior *Pax2* expression in the *Osr1* mutants is due to some partial rescue or redundancy by *Osr2*, or is completely cell autonomous and independent of Osr1 function remains to be determined.

Intermediate mesoderm specification along the mediolateral axis

The activation of the Pax2/Pax8 expression domain might be the first indication that the LPM and the IM have assumed separate fates. This activation appears to depend on BMP signals that come from the lateral plate or from the overlying ectoderm, and on opposing signals from the somite. A model for IM fate commitment (Fig. 1A) was first proposed by James and Schultheiss (James and Schultheiss, 2003; James and Schultheiss, 2005) after a series of

embryonic manipulations in the chick, in which low concentrations of BMPs activated IM-specific genes, whereas higher concentrations activated lateral plate markers. Thus, the source of BMPs is more lateral, and probably dorsal. Ectopic BMPs can shift the position of the IM, even transforming more paraxial mesoderm into an IM phenotype. These data are consistent with earlier observations that BMPs could replace the overlying surface ectoderm as inducers of the primary nephric duct within the IM (Obara-Ishihara et al., 1999).

The dorsolateral BMP signals may be opposed by as yet unidentified negative signals emanating from the somites (Fig. 1A), as first proposed by Mauch et al. (Mauch et al., 2000). Other secreted signals that are known to promote IM marker gene expression and kidney development are activin and retinoic acid. In the frog, activin and retinoic acid can induce *Lhx1* expression in animal caps and expand the pronephric field (Osafune et al., 2002; Taira et al., 1992). In mouse embryonic stem (ES) cells, activin and retinoic acid can increase the expression of IM markers when added to embryoid bodies and can promote differentiation along the renal epithelial lineage (Kim and Dressler, 2005; Vigneau et al., 2007). In a new study by Preger-Ben Noon et al. (Preger-Ben Noon et al., 2009), activin also induced *Lhx1* expression in the chick embryo along the entire body axis, but failed to induce *Pax2* expression more anteriorly. Indeed, this study found no evidence for a BMP- or activin-gradient model when using antibodies for phosphorylated Smad (P-Smad) proteins as read outs of signaling. Yet several caveats here must be considered. First, P-Smad detection is difficult and antibody specificity for immunostaining is always an issue. Second, BMPs are also known to signal through alternative, Smad-independent pathways, including the p38 and the c-Jun N-terminal kinase pathways (Derynck and Zhang, 2003), the latter of which has been linked to Pax2 phosphorylation (Cai et al., 2003; Cai et al., 2002) and to early kidney progenitor cell differentiation in vitro (Cai et al., 2003; Cai et al., 2002; Osafune et al., 2006).
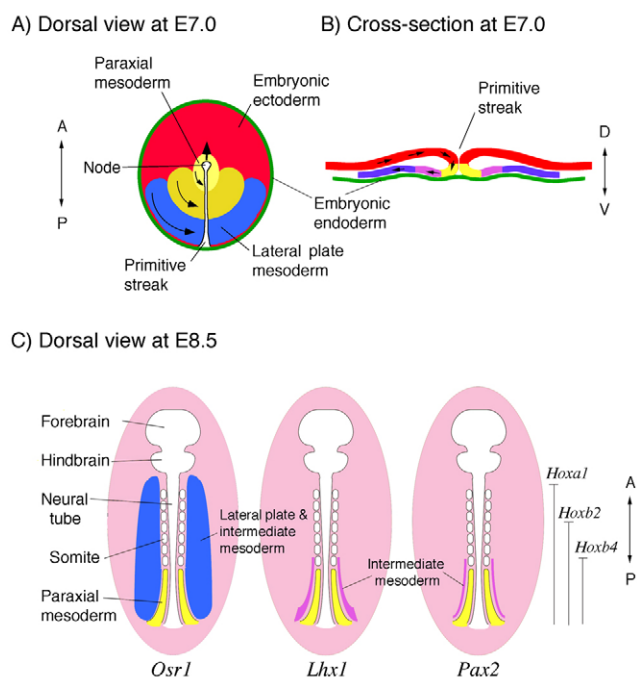
Fig. 2. Specification of the intermediate mesoderm. (A,B) During mouse gastrulation, cells of the epiblast ingress through the primitive streak. The more anterior and medial cells are fated to generate paraxial mesoderm (yellow), whereas more posterior and lateral cells make lateral plate mesoderm (blue). The intermediate mesoderm (IM, purple) is not well defined at this time. A is a dorsal view shown as a flat projection, as the mouse epiblast is really cup shaped; B is a posterior cross-section through the primitive streak. **(C)** Schematic of a flattened E8.5 mouse embryo from the dorsal side. The expression of specific gene products demarcates the IM, which has an anterior border at approximately the sixth somite. *Osr1* expression (blue) extends more anterior and more laterally than does *Lhx1* expression (purple), which becomes restricted to the IM. The *Pax2* (and *Pax8*) expression domain (purple) also marks the IM and extends caudally. The anterior boundaries of selected Hox genes are marked. The *Lhx1* and *Pax2* expression domains correspond to the anterior boundary of the Hox4 paralogous group.

If combinations of BMPs, activins and midline signals emanating from somites act in concert to specify gene expression patterns along the mediolateral axis, then manipulating such secreted factors, their receptors and their inhibitors ought to reveal patterning defects and transformations within the developing mesoderm. Unfortunately, experimental manipulation of BMPs and activins is compounded by their broad expression and use in many other developing tissues in the post-gastrulation embryo. The development of IM and LPM promoters for driving specific activators in vivo, such as the Tet-on system or Cre recombinase specific activation or repression, would facilitate the creation of both gain- and loss-of-function mutations to delineate signaling pathways for mesodermal patterning. The question then remains as to what it is that is unique about the mesoderm that facilitates responses to BMPs that are different from the responses they elicit in other tissues, such as in the neural ectoderm or the limb bud, for example.

Nevertheless, taken together these recent findings suggest that signals emanating from the medial tissues, neural tube and somites compete with signals from the more dorsolateral surface ectoderm to determine the activation of IM-specific genes, perhaps in a concentration-dependent manner. How these signals are integrated and whether they truly oppose each other remains to be determined. Still, these types of opposing signals would not be new or unique to the kidney morphogenetic field, as BMP signaling and its inhibition has long been one of the best characterized examples of axial patterning in frogs and flies (Garcia Abreu et al., 2002). However, the mediolateral axis is only one body axis. The developing IM must also be specified along the anteroposterior (AP) axis, as is evident from the morphological differences between mesonephric and metanephric tissues.

Intermediate mesoderm specification along the AP axis

A distinguishing feature of the mammalian kidney is that it forms along the length of the body axis in a manner reminiscent of its evolutionary history. Although many of the same genes are expressed along the entire IM, the morphological structures derived from different regions along the axis are unique. Regional specification along the anteroposterior axis has long been within the realm of the Hox genes, the functions of which in axial skeleton, and central and peripheral nervous system patterning are well established (Deschamps and van Nes, 2005; Guthrie, 2007; Hunt and Krumlauf, 1992; Kiecker and Lumsden, 2005; Trainor and Krumlauf, 2001). However, the roles of Hox genes in mesoderm regionalization are less well characterized. In this regard, the IM and the developing kidney are proving to be useful systems for investigating Hox function.

The problem with mammalian Hox genes and conventional genetic analyses has been the functional redundancy that exists among paralogous groups within the four Hox gene clusters, such that most phenotypes are evident only if multiple genes of a group are deleted (Wellik, 2007; Zakany and Duboule, 2007). Still, the necessity for Hox genes in the developing mouse metanephric kidney was first alluded to by Patterson et al. (Patterson et al., 2001) in compound mutants for *Hoxa11* and *Hoxd11*, which exhibited metanephric branching defects and hypoplasia. Such kidney defects are even more severe if all three mouse Hox11 paralogous genes are deleted, and include complete agenesis and lack of ureteric bud outgrowth (Wellik et al., 2002). These posterior defects did not affect genes such as *Pax2* or the Wilms' tumor suppressor gene *Wt1*, the expression of which is found along the entire AP axis within the IM. Rather, Hox11 genes are necessary for the expression of more posterior markers that delineate the metanephric mesenchyme only. Among the more posterior genes affected by the loss of Hox11 function are *Gdnf* (glial cell line derived neurotrophic factor) and *Six2* (the sine oculis-related homeobox 2 gene), which demarcate the metanephric mesenchyme by E10.5. If the Hox11 genes are important for differentiating the metanephric mesenchyme from more anterior IM derivatives, then altering the pattern of Hox genes might induce a regional shift in identity. Such an approach was developed by Mugford et al. using an *Osr1* driver to activate *Hoxd11* in the more anterior IM, encompassing the normal *Osr1* domain (Mugford et al., 2008a). This ectopic *Hoxd11* expression partially transformed mesonephric tubules to a more metanephric phenotype, as based on the expression of marker genes exclusive to metanephric tubules, such as calbindin 3 (also known as S100g), suggesting that Hox11 genes are necessary for specifying the metanephric identity from more anterior or mesonephric IM.

In the most anterior IM and at earlier stages, Hox genes also appear to set the boundary for competence to respond to the mediolateral IM patterning signals described in the previous section (Fig. 2). The signals that induce the expression of IM-specific genes,

such as *Lhx1* and *Pax2*, are present along the entire body axis, but the IM-specific markers are only induced posterior to the sixth somite in the chick embryo, suggesting that only mesoderm formed posterior to this region is competent to respond and make IM (Barak et al., 2005). This anterior boundary of prospective IM is coincident with the anterior expression boundary of Hox4 paralogs. Remarkably, by using retinoic acid to shift the *Hoxb4* expression domain more rostrally or by just overexpressing *Hoxb4* with plasmids in the chick embryo, the boundary for IM competence was shifted more anteriorly (Preger-Ben Noon et al., 2009). These data provide strong evidence for the existence of an AP patterning code within the mesoderm that is regulated by specific Hox gene paralogous groups.

How is the AP patterning code translated into a biological response along the mesoderm? Genetic analyses in mice have not provided many clues, although there are some examples of AP patterning shifts in some mutants. For example, the *Foxc1* and *Foxc2* genes, of the forkhead winged helix family of transcription factors, appear to suppress the anterior expression of *Gdnf*, thus restricting the expression to the metanephric mesenchyme at E10.5 (Kume et al., 2000). Whether the Foxc proteins are co-factors and interact directly with Hox proteins remains to be determined. However, recent examples of coordinated *Foxp1* and Hox gene action in the specification of spinal cord motoneurons would lend support to such an idea (Rousso et al., 2008). Direct interactions of Hox proteins with other IM specific transcription factors, specifically Eya1 and Pax2, do seem to be necessary for *Six2* gene activation in the metanephric mesenchyme, suggesting that regionalization of specific IM compartments depends upon the intersection of expression domains between the mediolateral factors, such as Pax2, Pax8 and Lhx1, and the AP factors (Gong et al., 2007).

Thus, it appears that a specific pattern of Hox gene expression predisposes the mesoderm to respond to IM inductive signals at the anterior boundary, which initiates expression of *Lhx1*, *Pax2* and *Pax8* along the entire body axis caudal to the sixth somite. By contrast, a posterior combination of Hox genes, consisting primarily of the Hox11 paralogous group, is needed to activate genes such as *Gdnf* and *Six2*, and to distinguish the metanephric mesenchyme from more anterior mesonephric tissue. The question then remains whether the same secreted signaling factors, namely activin and retinoic acid, that induce the IM at the anterior pole also induce posterior IM. If so, then Hox genes might alter the response to inducing factors by epigenetic means, essentially by altering the chromatin structure and thus the accessibility of anterior and posterior targets of the inducing signal. An alternative and more direct mechanism might involve Hox proteins as direct co-factors for the activation or repression of anterior and posterior specific targets. These questions are relevant not just for kidney development, but for understanding the biochemical functions of Hox proteins in general, a problem that is still unresolved and that is complicated by the lack of Hox DNA-binding specificity and the functional redundancy among paralogous genes. However, once the posterior IM is ultimately specified, its development proceeds along a markedly different path compared with that of the more anterior IM as the adult kidney is formed.

The ureteric bud

The most compelling argument that the posterior region of the IM is somehow different from more anterior regions is the unique ability of the posterior nephric duct and the surrounding mesenchyme to generate the ureteric bud, an epithelial diverticulum that invades the metanephric mesenchyme to initiate adult kidney development. The
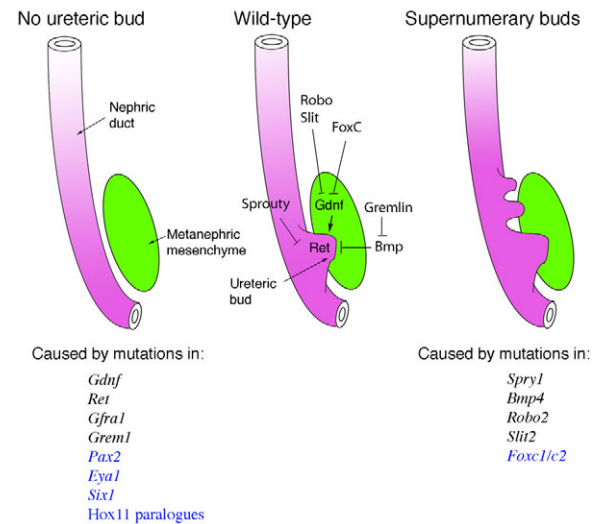
Fig. 3. Signals that promote or suppress ureteric bud outgrowth. The outgrowth and invasion of the ureteric bud (UB) from the nephric duct initiates metanephric kidney development. In a wild-type embryo (middle), Gdnf secretion from the metanephric mesenchyme activates the receptor tyrosine kinase Ret, via the co-receptor Gfra1, and promotes UB outgrowth and invasion. Mutations in various regulatory genes can generate two phenotypes, UB ablation (left) or the induction of supernumerary, ectopic UBs (right). Genes encoding signaling proteins are listed in black, those that encode transcription factors are in blue. Not all mutant phenotypes are completely penetrant; for example, *Ret* and *Gdnf* mutant mice often show remnants of the UB. Genetic analyses indicate that multiple extracellular inhibitors of Gdnf signaling exist, such as BMPs and Robo/Slit, and an inhibitor of BMPs called gremlin, which is thus an activator of Gdnf signaling. Intracellular inhibitors of Ret signal transduction include Sprouty (Spry1), which may also limit Fgfr signaling. The nuclear factors Foxc1 and Foxc2 act to restrict Gdnf expression to the posterior region, whereas Pax2, Eya1, Hox11 and Six1 proteins all are needed for Gdnf expression.

signals that drive ureteric bud outgrowth have been well studied over the years (Fig. 3). Instrumental in the regulation of ureteric budding is the receptor tyrosine kinase Ret, the secreted neurotrophin Gdnf, and the membrane-anchored co-receptor Gfra1 (Gdnf family receptor alpha 1) (Costantini and Shakya, 2006). However, it has become clear that there are many modifiers of the Gdnf/Ret signaling pathway that regulate precisely the position and number of buds, and the subsequent branching morphogenesis of the ureteric bud epithelia. For example, a complex network of inhibitors restricts Gdnf/Ret signaling to a region of the nephric duct such that ectopic ureter budding is suppressed (Fig. 3). Bmp4 is expressed in the mesenchymal cells that surround the nephric duct, but is not expressed in the metanephric mesenchyme, and inhibits Gdnf/Ret signaling. BMP signaling itself is also blocked by the BMP inhibitor gremlin (Grem1), which is expressed in the metanephric mesenchyme and which blocks the BMP-dependent repression of Gdnf/Ret signaling (Michos et al., 2007). The transmembrane protein Slit2 (slit homolog 2) and its receptor Robo2 (roundabout homolog 2), which are homologous to the *Drosophila* proteins that provide axon guidance cues, also repress ectopic ureteric bud outgrowth and act on the metanephric mesenchyme to prevent *Gdnf* expression from extending rostrally (Grieshammer et al., 2004). Within the ureteric bud epithelial cells, the cytoplasmic protein Spry1 (sprouty homolog 1) appears to limit the intensity or duration

of Ret signaling, as *Spry1* mouse mutants develop ectopic ureter buds, a phenotype that can be suppressed by a reduction of *Gdnf* gene dosage (Basson et al., 2005; Basson et al., 2006).

How Ret activation has an impact on cell movement and proliferation is still not entirely clear, in part because of the large number of tyrosine residues that are phosphorylated on the Ret cytoplasmic domain, its potential for acting as a dock for many different second messenger proteins, and the difficulty in pursuing biochemical analysis in small embryonic tissues. A number of studies point to the activation of phosphatidylinositol 3-kinase (PI3K) and its target, the protein kinase AKT, in response to Gdnf-mediated Ret activation (Besset et al., 2000; Tang et al., 2002). In response to chemotactic agents, PI3K is activated at the leading edge of migrating cells to promote lamellipodia formation, extension and cell movement, whereas the lipid phosphatase PTEN (phosphatase and tensin homolog), which dephosphorylates the substrates for PI3K, is located at the trailing edge (Funamoto et al., 2002; Kolsch et al., 2008). In kidney organ cultures, inhibition of PI3K completely blocks ureteric bud outgrowth, suggesting that ureteric bud epithelial cell migration is essential for invasion of the metanephric mesenchyme (Tang et al., 2002). In vivo, the deletion of the PTEN phosphatase in the ureteric bud epithelia also leads to abnormal branching and patterning defects, consistent with a role for PI3K and

PTEN in shaping the ureteric bud by counteracting the effects of PI3K (Kim and Dressler, 2007). However, cell movement is not enough to drive invasion, as localized proliferation and extension must contribute to the growing bud tip. Other downstream effectors that are likely to transduce Ret signaling include the mitogen activated protein kinases (MAPKs), the inhibition of which also leads to branching defects (Fisher et al., 2001; Watanabe and Costantini, 2004). Mutations to specific tyrosine residues in different Ret isoforms indicate that at least two important docking sites for intracellular second messengers, such as Grb2/Grb7 and Shc, map to Y1015 and Y1062 of Ret and activate the PI3K and the MAPK pathways during kidney development (Jain et al., 2006; Wong et al., 2005). Taken together, the data point to the existence of multiple signaling pathways downstream of activated Ret that coordinate the proliferation and cell migration of ureteric bud epithelial cells, yet still maintain the integrity of the bud as an epithelial structure.

Despite all this complexity, the Ret pathway is not the only promoter of ureteric bud outgrowth as a significant proportion of Ret mutant kidneys still exhibit a rudimentary bud (Schuchardt et al., 1996). The development of the Hoxb7-Cre driver strain of mice has greatly facilitated the analysis of genes with pleiotropic developmental effects by enabling genes to be specifically deleted in the ureteric bud epithelium. The use of this Cre strain to delete the
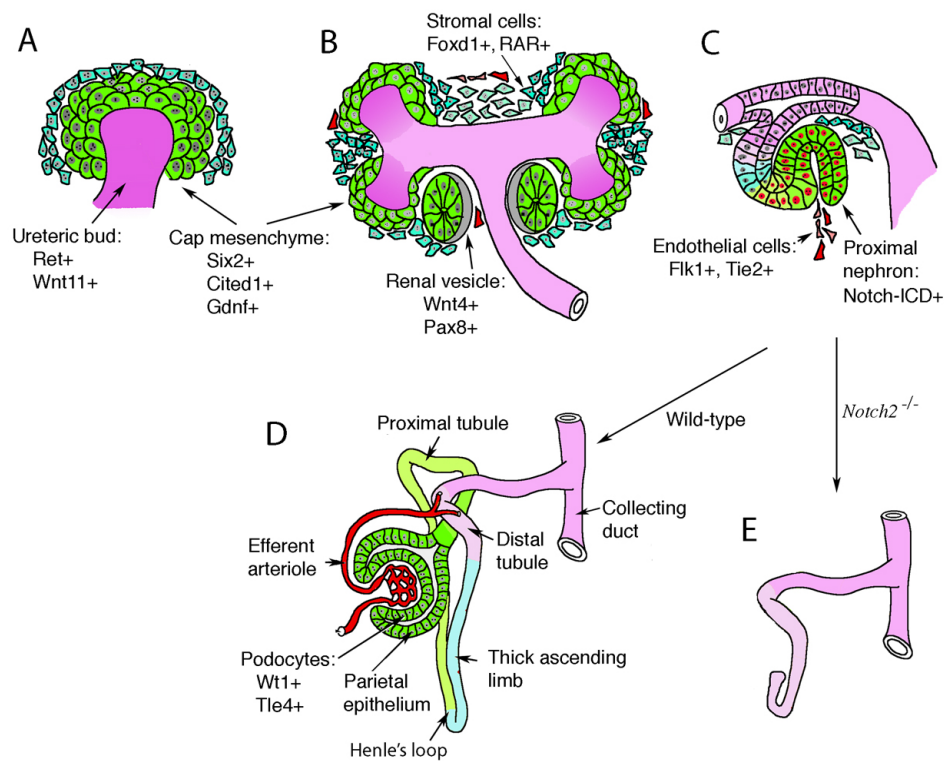
Fig. 4. Nephron development and cell lineages. Invasion of the metanephric mesenchyme (green, MM) by the ureteric bud (purple, UB) provides inductive signals that initiate nephrogenesis. (**A**) UB invasion induces MM cells to condense around the UB tips at E11.5 of mouse development. These so-called cap mesenchymal cells express a unique combination of markers (Six2, Gdnf, Cited1) and define a stem cell population. (**B**) Cap mesenchyme polarizes into a primitive epithelial sphere, the renal vesicle, coincident with the expression of additional markers, such as Wnt4 and Pax8. Cells in the metanephric mesenchyme that do not aggregate at the bud tips express Foxd1and the retinoic acid receptors (RARs) and mark the stromal population. (**C**) The renal vesicle fuses to the ureteric stalk, which forms the collecting ducts, and generates an S-shaped body with a proximal and distal cleft. The more proximal cleft is infiltrated by endothelial cells and forms the glomerular tuft. The proximal portion of the S-shaped body activates the Notch pathway, as seen by the presence of the cleaved Notch intracellular domain (ICD). (**D**) The nephron begins to take shape as glomerular development proceeds and the more proximal tubules elongate and grow towards the medulla to form the descending and ascending limbs of the loop of Henle. (**E**) Notch signaling is essential for the proximodistal patterning of the nephron, as *Notch2* mutations delete all proximal cell types and structures (Cheng et al., 2007).

fibroblast growth factor receptors Fgfr1 and Fgfr2 has demonstrated that *Ffgr2* but not *Fgfr1* functions in the ureteric bud epithelium to fine tune the pattern of branching morphogenesis and to determine the size of the kidney and the number of nephrons (Zhao et al., 2004). Similarly, deletion of the receptor tyrosine kinase Met, the ligand of which is hepatocyte growth factor (HGF), also restricted branching morphogenesis, kidney size and nephron number (Ishibe et al., 2009). These results also raise the possibility that inhibitors of Ret signaling can inhibit other tyrosine kinases important for branching morphogenesis; for example, the Sprouty1 protein is known to suppress Fgfr signaling (Aranda et al., 2008). This further increases the complexity of signaling in the ureteric bud epithelium and makes interpretations at the biochemical level difficult.

At this point, we can summarize ureteric bud outgrowth as a complex phenomenon that requires both positive and negative signaling to drive cell movement and proliferation of epithelial cells at a precise position along the nephric duct. The proteins involved include secreted signaling factors and tyrosine kinase receptors, many of which are also used for other chemotactic processes, such as axon guidance and directed cell migration.

The metanephric mesenchyme

The metanephric mesenchyme is the anlagen of the adult kidney, and as a result has received the most attention over the years. Once the bud invades the metanephric mesenchyme it provides a permissive signal that stimulates the condensation of metanephric mesenchymal cells around the ureteric bud tips. This step begins the polarization of the mesenchyme to generate the epithelial cells of the nephron (Fig. 4). The identity of the signals emanating from the ureteric bud tip has been the subject of much investigation. The most compelling data to date indicate that Wnt proteins are the primary initiators of condensation. Multiple Wnt genes are expressed in the ureteric bud and the stalk, but genetic ablation experiments show that *Wnt9b* encodes the only Wnt protein that meets all of the criteria for the inducer of the metanephric mesenchyme (Carroll et al., 2005).

Wnt9b-expressing cells can mimic inductive signals and promote mesenchymal aggregation in vitro, whereas loss of *Wnt9b* in vivo prevents metanephric mesenchymal aggregation but has no effect on the initial budding and branching of the ureteric epithelium. The initial Wnt inductive signal transduction is canonical, as it can be mimicked by activation of β-catenin, although this must be attenuated in the early condensates as constitutively active β-catenin inhibits mesenchymal aggregates from progressing to polarized epithelia (Park et al., 2007).

Prior to induction at E10.5, the metanephric mesenchyme expresses a unique set of marker genes, many of which are known to regulate important events in early kidney development (see Table 1). By E11.5, the metanephric mesenchyme has been invaded by the ureteric bud epithelium and condensations of mesenchymal cells around the ureteric bud tips are visible (Fig. 4A). These condensates, now referred to as the cap mesenchyme, are the progenitor cells of the nephron epithelia and are themselves surrounded by stromal cells, which remain mesenchymal and migrate towards the interstitium (Fig. 4A,B). By E13.5, the S-shaped bodies derived from the cap mesenchyme become infiltrated by endothelial precursors to form the glomerular tuft (Fig. 4C), which consists of the capillary loops, the mesangium, the glomerular basement membrane and the podocyte cells.

One issue that has plagued investigators in this field is the pluripotency of the metanephric mesenchyme. Are these the stem cells of the kidney or are the metanephric mesenchymal cells a heterogenous mixture of epithelial, stromal and endothelial precursors? Several recent papers have provided new insights into the specification of early cell lineages from the posterior nephric region. Instrumental in these analyses was the development of modern in vivo genetic cell lineage tracing techniques using the Cre-lox recombinase system, which has replaced the more traditional diI or retroviral cell-labeling methods. In one study, *Osr1*-positive cells appeared to be capable of making either stromal or epithelial precursors prior to E10.5 (Mugford et al., 2008b), consistent with

Table 1. Genes that regulate early kidney development and cell lineages

Gene	Expression	Mutant phenotype
Early mesodermal regionalization		
Osr1	LPM, IM	Posterior nephric structures fail to develop (James et al., 2006; Tena et al., 2007)
Lhx1	LPM, ND	No nephric duct, no kidneys (Shawlot and Behringer, 1995; Tsang et al., 2000)
Pax2	IM, ND	No mesonephric tubules, no metanephros (Torres et al., 1995)
Pax2/Pax8	IM	No nephric duct, no kidneys (Bouchard et al., 2002)
Metanephric development		
Wt1	IM, MM	Fewer mesonephric tubules, apoptosis of mesenchyme (Kreidberg et al., 1993)
Foxd1	MM, SC	Developmental arrest, few nephrons, limited branching (Hatini et al., 1996)
Hox11	MM	No metanephros (Wellik et al., 2002)
Eya1	MM	No induction of mesenchyme (Xu et al., 1999)
Six1	MM	No UB, no induction (Xu et al., 2003)
Six2	MM, CM	Premature differentiation of CM, no self-renewal (Self et al., 2006)
Sall1	MM	UB invasion but no branching, no induction (Nishinakamura et al., 2001)
Wnt9b	UB	Failure to induce the MM (Carroll et al., 2005)
Nephron patterning		
Wnt4	CM	No polarization of CM, developmental arrest (Stark et al., 1994)
Fgf8	MM, CM	Cell death, few renal vesicles, developmental arrest (Grieshammer et al., 2005)
Bmp7	UB, MM	Developmental arrest post-induction, some branching, few nephrons (Dudley et al., 1995; Luo et al., 1995)
Notch2	RV, SB	Deletion of proximal nephron (Cheng et al., 2007)
Tcf21 (*Pod1*)	SC, PC	Poorly differentiated podocytes (Quaggin et al., 1999)
Pdgfr	PC	No vascularization of glomerular tuft (Soriano, 1994)

CM, cap mesenchyme; IM, intermediate mesoderm; LPM, lateral plate mesoderm; MM, metanephric mesenchyme; ND, nephric duct; PC, podocyte cells; RV, renal vesicle; SB, S-shaped body; SC, stromal cells; UB, ureteric bud.

the broad expression of pattern *Osr1* in the IM and the LPM. Yet once the metanephric mesenchyme is induced, the *Osr1*-positive population appears to make only epithelial cells, suggesting that the stromal progenitor cells have now turned off *Osr1* and are a separate lineage. The metanephric stromal cells, which express many genes not found in the cap mesenchyme, including *foxd1*, the retinoic acid receptors, and transcription factor 21 (*Tcf21*, also known as *Pod1*), are necessary for providing signals to promote epithelial cell survival and proliferation (Dudley et al., 1999; Hatini et al., 1996; Levinson et al., 2005; Mendelsohn et al., 1999). Recent cell lineage tracing experiments in the chick embryo (Guillaume et al., 2009) suggest that a large population of metanephric stromal cells is derived from paraxial mesoderm and that few arise from the IM. Taken together, the data suggest that stromal cells and epithelial precursors share a common *Osr1*-positive lineage before the onset of metanephric induction. However, after E11.5, the stromal and cap-mesenchyme lineages become separate. Indeed, it is possible that new stromal precursor cells, which are *Osr1* negative and which derive from more paraxial mesoderm, might migrate into the metanephric mesenchyme after induction.

Although the timing of stromal specification is still not entirely clear, cell lineage tracing studies of the cap mesenchyme have revealed a self-renewing population that can be considered to be the epithelial stem cells of the nephron. Lineage tracing experiments in which Cre recombinase was driven by either the *Cited1* (Boyle et al., 2008) or *Six2* (Kobayashi et al., 2008) genes clearly demonstrated that cap mesenchyme is pluripotent with respect to epithelial cell types, as its derivatives include glomerular, proximal tubular and distal tubular epithelia. As such, the cap mesenchyme must proliferate and generate cells of the renal vesicles, the progenitors of the nephrons, whilst also repopulating the aggregates around the tips of the branching ureteric buds for the next round of nephron formation. The decision of whether to differentiate or self-renew requires *Six2*, as its loss leads to precocious metanephric mesenchyme differentiation (Self et al., 2006). Rather than forming renal vesicles in particular positions under the ureteric bud tips, *Six2* mutant mouse embryonic kidneys have epithelial structures all along the T-shaped ureteric bud, which results in exhaustion of the cap mesenchyme population and in rudimentary kidneys. All the cap mesenchyme has presumably seen the inductive Wnt9b signals from the ureteric bud tips, otherwise the cells would not aggregate around the ureteric bud tips (Carroll et al., 2005; Kobayashi et al., 2008). However, cap mesenchyme requires the downregulation of canonical, β-catenin-mediated Wnt signaling (Marose et al., 2008; Park et al., 2007) and the expression of Wnt4 to become polarized into renal vesicles (Stark et al., 1994). Thus, one function of *Six2* could be to suppress the intrinsic Wnt4 signals that emanate from induced cap mesenchyme and that promote epithelial polarity.

Additional secreted signaling molecules that are essential for mesenchyme polarization include Fgf8 and Bmp7. Loss of *Fgf8* results in depletion of the cap mesenchyme and significant cell death in the peripheral, nephrogenic zone (Grieshammer et al., 2005). Using a Pax3-Cre driver to conditionally delete *Fgf8* in the mouse metanephric mesenchyme inhibited *Wnt4* and *Lhx1* expression, but not *Pax2* expression. Despite the absence of detectable Wnt4, some cells did progress to the renal vesicle stage at early times after induction, but by approximately E14.5 significant cell death prevented any further development and mesenchymal aggregate formation. In this case, it is difficult to say exactly why a limited number of renal vesicles could still form if *Wnt4* and *Lhx1* were not expressed. Perhaps the Cre-mediated Fgf8 deletion was not complete at early times and the residual level of Wnt4 expression

was below the level of detection. Bmp7 deletion also led to developmental arrest, but metanephric mesenchyme cells induced at E11.5 did progress to the polarized epithelial stage and were able to generate tubules (Dudley et al., 1995; Luo et al., 1995). It appears that *Bmp7* deletion also depleted the cap mesenchyme stem cell population. These data are consistent with a role for Fgfs and Bmp7 in providing survival signals for the metanephric mesenchyme, perhaps by expanding the stromal cell population that helps to support the cap mesenchyme (Dudley et al., 1999).

Six and Eya proteins are known to interact physically and genetically in other developing tissues (Kochhar et al., 2007; Kumar, 2009), yet *Eya1* mutations are completely recalcitrant to the inductive signals emanating from the ureteric bud and thus do not mimic the *Six2* mutant phenotype (Sajithlal et al., 2005; Xu et al., 2003). Instead, *Eya1* appears to interact with *Six1*, as both genes are essential for early metanephric mesenchyme specification and are associated with branchio-oto-renal (BOR) syndrome in humans carrying one mutant allele of either gene (Abdelhak et al., 1997; Kochhar et al., 2007). BOR syndrome is characterized by unilateral or bilateral renal hypoplasia, dysplasia or agenesis, in addition to cochlear defects and craniofacial fistulas.

In addition to the stroma and cap mesenchyme, the early metanephros also contains precursors of the vasculature. Angioblasts are integral to the development of the glomerular tuft, which depends on a precise level of vascular endothelial growth factor (VEGF) signaling (Quaggin and Kreidberg, 2008). However, these *Flk1*-positive angioblasts also signal to other cell types to propagate the inductive signals (Gao et al., 2005). A recent paper also described crosstalk in ureteric bud cells in culture, in which VEGF was able to promote the phosphorylation of Ret, perhaps accounting for some of the redundancy in the budding mechanism (Tufro et al., 2007).

Clearly induction of the metanephric mesenchyme by the ureteric bud is the crucial step in kidney development and leads to all subsequent differentiation. Induction appears to be sequential and requires at least two different Wnt proteins to begin the process of epithelial polarization. Many of the genes expressed in the metanephric mesenchyme prior to induction are needed in order to respond to Wnt signals, suggesting that the mesenchyme is already programmed to become renal tissue and just needs a permissive signal, not an instructive signal. Indeed, Saxen made this distinction early on merely by observing that any inducing tissue that could promote epithelial cell polarization of the mesenchyme always resulted in the differentiation of renal epithelia and not any other type of epithelia (Saxen, 1987).

Patterning of the nephron

The epithelial cells of the nephrons are derived from the cap mesenchyme that makes first the renal vesicle and then the S-shaped body (Fig. 4). The renal vesicle is a primitive epithelium with a basement membrane and lumen that is in close proximity to the ureteric bud stalk. By the S-shaped body stage, the renal vesicle has fused to the ureteric stalk to form a continuous epithelial tubule with a common apical lumen (Fig. 4C). The differential expression of cadherin genes has provided some early evidence for the patterning of the renal vesicle (Cho et al., 1998). However, recent large-scale expression screens coupled with three-dimensional reconstruction reveal significant differences in gene expression in the renal vesicle, along the proximodistal axis, with respect to the adjacent ureteric bud stalk, the progenitors of the collecting ducts (Georgas et al., 2009). These differences in gene expression are likely to underlie the regionalization of the vesicle into glomerular, proximal and

distal segments. Strikingly, the distal segments fuse to the prospective collecting ducts by the degradation of the epithelial basement membrane and the integration of distal renal vesicle cells into the prospective collecting tubules (Georgas et al., 2009).

The definitive proximodistal axis of the nephron is clear by the S-shaped body stage (Fig. 4). At the most proximal end are the precursors of the glomerular podocyte cells, the visceral glomerular epithelium. At the distal end, the S-shaped body has fused to the branching ureteric tree to form the collecting tubules. Until recently, little was known regarding the signals that specify this proximodistal axis and the different epithelial cell types that arise along the axis. Thus, one of the most significant discoveries in recent years is the role of the Notch pathway in proximodistal patterning of the S-shaped body (Cheng et al., 2007). Although Notch and its ligands Delta, Jagged and Serrate are known to specify neural cell fates by lateral inhibition in the fly eye (Blair, 1999) and the mouse immune system (Tanigaki and Honjo, 2007), the Notch pathway having a direct role in the regional specification of a tissue had not been previously reported. Kidneys from mice homozygous for a *Notch2* null allele have a complete absence of more proximal renal cell types, including the glomeruli and the proximal convoluted tubules (Cheng et al., 2007). These mutants have normal ureteric bud epithelial branching, and more distal derivatives form the renal vesicle. Conversely, the expression of an activated Notch intracellular domain in wild-type cap mesenchyme can transform more distal fates into more proximal fates. Similar results were observed in *Xenopus* following the ectopic expression of the downstream target of Notch, *Hairy/Enhancer of Split*, which can also induce more proximal fates along the developing pronephros (Taelman et al., 2006). In zebrafish, knockdown of either *Notch3*, its potential ligand Jagged 2, or its downstream effectors has demonstrated a role for Notch in differentially specifying the fates of transport epithelia from multiciliated cells, two different terminal cell types interspersed along the pronephric duct (Liu et al., 2007). This fate decision is somewhat akin to Notch-mediated lateral inhibition in the fly eye. Interestingly, these specific Notch knockdowns did not affect the single midline glomerulus in the zebrafish larvae pronephros, unlike the loss of Notch signaling in the mouse metanephric kidney. This apparent discrepancy is likely to reflect the unique origin of the zebrafish pronephric glomerulus, which does not require the same genetic components as the metanephric glomerulus. For example, loss of zebrafish *Pax2* results in ablation of the pronephric duct and tubules but not the midline glomerulus (Majumdar et al., 2000).

At the extreme proximal end of the nephron, there has been significant progress made in understanding the development of the glomerulus and the relationships between podocytes, the endothelial cells, and the mesangial precursor cells of the glomerular tuft (for a review see Quaggin and Kreidberg, 2008). I believe this is because of the clinical relevance of the glomerular filtration barrier and the numerous human genetic mutations that impact podocyte function, the integrity of the slit diaphragm, which forms the filtration barrier,
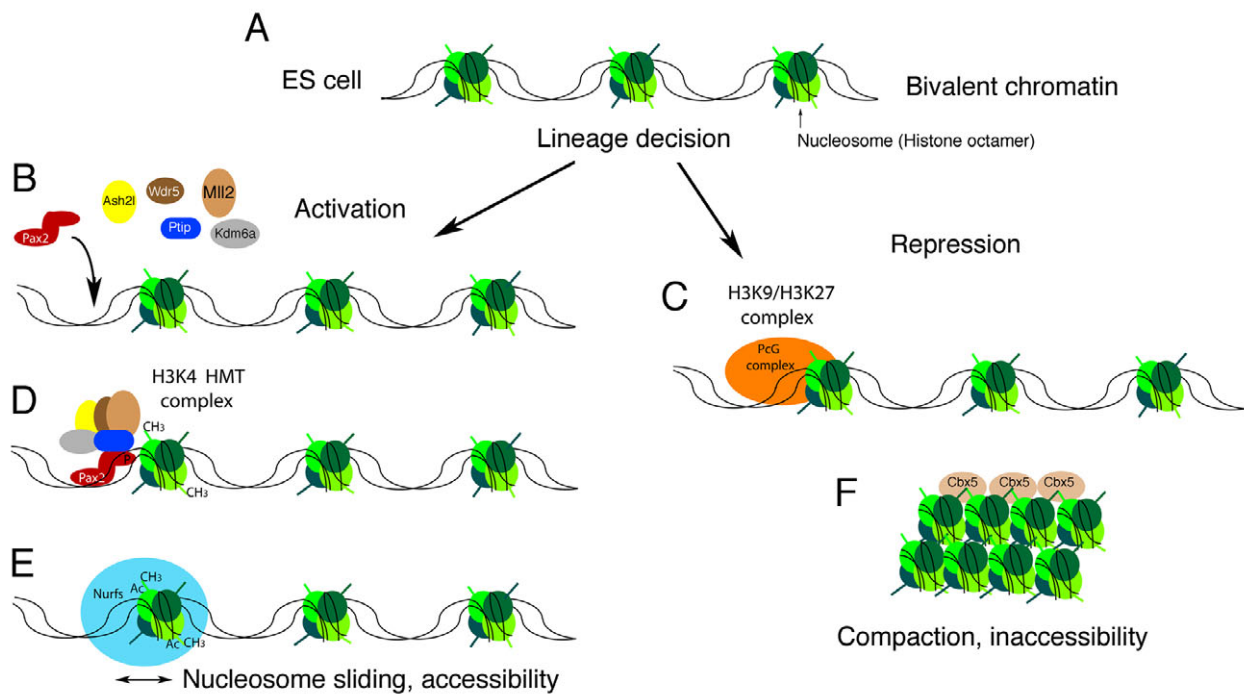
Fig. 5. A chromatin model for the epigenetic specification of cell lineages. As cells make lineage decisions, alterations in chromatin structure compartmentalize the genome into active and inactive domains. (**A**) In pluripotent embryonic stem (ES) cells, tissue-specific genes and developmental regulators are marked with a bivalent histone code that encompasses low levels of both positive and negative histone methylation marks. As cells become specified and their fates are restricted, cell-type specific DNA-binding proteins could provide locus specificity for the modification of chromatin into active (B) or repressed (C) domains. (**B**) During intermediate mesoderm specification, the Pax2/Pax8 proteins might interact with a histone H3K4 methyltransferase (HMT) complex (D) to prevent repression of kidney-specific genes by the polycomb group (PcG) complexes. (**E**) High levels of H3K4 trimethylation could then recruit nucleosome remodeling factors (Nurfs) that maintain the accessibility of genes and facilitate transcription. (**F**) Conversely, PcG-mediated methylation of histone H3 at K9 and K27 could recruit heterochromatin-binding proteins that compact DNA into tightly packaged, silent chromatin. Ac, acetyl; Ash2l, absent small or homeotic like 2; Cbx5, chromobox homolog 5; CH$_3$, methyl; Kdm6a, 4 lysine (K)-specific demethylase 6A; Mll2, mixed-lineage leukemia 2; P, phospho; Ptip, Pax trans-activation domain interacting protein; Wdr5, WD repeat domain 5.

and the stability of the glomerular basement membrane, which contains unique collagen and laminin chains. By contrast, the mechanisms that differentiate the cell types along the axis of the nephron have not been investigated, in large part because molecular markers that distinguish these early decision making events have been unavailable. However, this is issue is being addressed through large-scale expression screens undertaken by the GenitoUrinary Development Molecular Anatomy Project (www.gudmap.org), which has found unique expression signatures for all specific anatomical structures within the developing metanephric kidney (Brunskill et al., 2008; McMahon et al., 2008). Thus, new and better reagents coupled with improved anatomical descriptors are at hand for tackling these outstanding issues of terminal epithelial sub-type specification and function.

Integrating patterning, lineage specification, and gene regulation

The developing kidney is useful as a model system for studying epithelial cell specification, mesenchymal-epithelial interactions, and complex patterning events in three dimensions. We know much about which genes are important for morphogenesis and which genes affect the downstream expression of known markers. However, despite all of this available knowledge, it is still difficult to ascribe biochemical functions to many of the proteins that we know are essential for kidney development. In fact, many of the genes that regulate early kidney development were not identified from their functions in the kidney at all. Some genes, such as *Osr1*, *Pax2*, *Pax8*, *Eya1*, *Six1* and *Six2*, were identified purely by sequence homology to regulatory genes in the fly. Other genes, such as *Gdnf* and *Wnt4*, were assigned kidney functions once they were mutated in mice and the consequent kidney phenotypes became evident. Particularly for the intrinsic, cell-autonomous nuclear factors, such as the Pax, Eya and Hox families, how they function to specify early patterns and renal cell lineages remains mysterious. Because many of the early marker genes encode DNA-binding proteins, it is assumed that they regulate some aspect of transcription, presumably of other kidney-specific genes. How do proteins like Pax2/Pax8, Lhx1, Eya1 and Osr1 talk to the transcription regulatory machinery to establish the early IM and the kidney epithelial lineages? The standard tools of genetics are unlikely to yield all the answers because the biological readouts, i.e. the failure to develop, are not very specific. These problems are not unique to kidney development. To understand the biochemical function of a nuclear protein, its interaction with DNA and with other cellular factors must be defined within the appropriate context. Yet, the context is often inaccessible to standard biochemical purification or manipulation because cells corresponding to the progenitor state in which these proteins function are not available as stable cell lines. However, new ways of thinking about developmental competence are emerging from the rapidly progressing fields of epigenetics and chromatin biology, which can impact how we think about early gene regulation and cell lineage restriction (Fig. 5).

The restriction of cell fate is a sequential process during development that implies a heritable imprint on the genome of a progenitor population. These types of epigenetic imprints are within the realm of the polycomb and trithorax family of genes, the protein products of which are involved in establishing and maintaining patterns of histone methylation on chromatin (Kouzarides, 2007; Ringrose and Paro, 2007). The histone octamer is the primary protein component of the nucleosome and its modification dramatically impacts the structure of chromatin. Many of the most interesting developmental regulatory genes in pluripotent embryonic

stem (ES) cells have a bivalent pattern of histone modification, with low levels of both positive and negative epigenetic marks that are then resolved into active or inactive marks upon differentiation along particular lineages (Azuara et al., 2006; Bernstein et al., 2006; O'Neill et al., 2006). These findings imply that there must be cell lineage-determining factors that control the locus and tissue specificity of histone modifications during early developmental decision making. In the developing eye, the Pax6 proteins were proposed to have such early lineage decision-making potential in the fly and the mouse (Chow et al., 1999; Quiring et al., 1994). In biochemical studies, the Pax2 protein was shown to promote histone H3K4 methylation at a Pax DNA-binding sequence by recruiting a ubiquitous adaptor protein, called PTIP (Pax transactivation domain interacting protein; also known as Paxip1), and a mammalian trithorax-like histone methyltransferase complex (Patel et al., 2007). Consistent with this idea, the PTIP protein is also conserved in flies where it regulates the expression of segmentation genes and global levels of H3K4 trimethylation (Fang et al., 2009). These data suggest that Pax2 might provide some locus and tissue specificity to the imprinting of kidney-specific epigenetic fate by partitioning the genome of the IM into active and inactive domains that are unique for the renal lineage (Fig. 5). Whether H3K4 trimethylation promotes gene expression or merely inhibits polycomb-mediated repression still needs to be clarified, as genetic evidence suggests that repression is the default state in the absence of trithorax-mediated derepression (Klymenko and Muller, 2004). In any case, more definitive proof of this concept awaits better technology that could characterize chromatin modifications at single genes in a spatial and temporal manner, in small numbers of cells, during development.

Development and disease

One advantage to studying organ development in a mammalian system is the potential for cross-referencing with human genetics, pathology and clinical medicine. In many cases, human mutations in kidney developmental control genes have been described and the resulting clinical phenotypes illustrate important functions. For example, the BOR syndrome is associated with mutations in either the *Eya1* gene (Abdelhak et al., 1997) or the *Six1* gene (Ruf et al., 2004) and thus strongly suggests that these proteins interact and function in similar biochemical pathways. The embryonal cancer syndrome Wilms' tumor led to the discovery of the *WT1* gene, a critical survival factor for metanephric mesenchyme in development and for glomerular podocytes in adults (Guo et al., 2002; Kreidberg et al., 1993). The discovery of nephrin (Kestila et al., 1998), which accounts for congenital nephrotic syndrome of the Finnish type, was instrumental in defining the podocyte slit-diaphragm and the glomerular filtration barrier. The role of the primary cilia in epithelial cell polarity, in defining the axis of cell division, and in epithelial cyst formation was deduced primarily through studies on polycystic kidney disease (PKD) and the human genes responsible (Boletta and Germino, 2003). This convergence of mouse and human genetics really underscores the importance of developmental genetic pathways and provides significant insight into the origins of certain diseases.

The concepts of developmental biology are also relevant for understanding adult kidney injury and repair. In humans, acute renal failure is a common result of nephrotoxicity or ischemia. Therapies for acute renal failure are lacking and, despite dialysis, the morbidity has not fallen much below fifty percent. In animal models of renal injury, subsets of proximal tubules cells are killed, but if the time of ischemia or the dose of nephrotoxins is carefully titrated, the injured

kidneys will recover and repopulate the damaged tubules. The origin of these regenerating proximal tubule cells has been studied in some detail. Although the population of embryonic renal stem cells has now been well defined as the *Six2*-positive cap mesenchyme, the issue of whether adult renal stem cells exist to repopulate the kidney after injury has been controversial. The idea of bone marrow-derived mesenchymal stem cells as a source of renal epithelia in regenerating tissue did gain some popularity when the adult stem cell field was still in its infancy (Kale et al., 2003; Lin et al., 2003). However, more recent studies refute these earlier findings, as more careful analyses show the presence of few bone marrow-derived epithelial cells in regenerating kidneys (Duffield et al., 2005; Lin et al., 2005; Szczypka et al., 2005). More recent lineage-tracing methods demonstrate that adult regenerating proximal tubule cells are derived primarily from pre-existing, surviving proximal tubular epithelia, which themselves were derived from the *Six2*-positive cap mesenchyme (Humphreys et al., 2008). What promotes these surviving epithelial cells to enter the mitotic cycle and repopulate the damaged tubules? The reactivation of developmental genes such as *Pax2* has been described (Imgrund et al., 1999), but the stimulus for this expression is unknown. If the signals that reactivate the developmental programs could be defined after injury, the ability to enhance regeneration would greatly impact treatment options for acute renal failure.

Conclusions

The kidney is not only a good model for complex organ development it is also of great clinical interest. What can be gleaned from developmental studies might ultimately provide significant insight into disease mechanisms, and may pave the road for new and improved therapies. Through the power of mouse genetics and the systematic screening of thousands of genes (www.gudmap.org), we now have resources available to analyze kidney development in a more holistic manner. The progress made and the resources available should stimulate more young investigators to recognize this field as an opportunity, not only for addressing basic problems in development and epithelial cell biology, but also as a means to impact the health of our communities.

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