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Induction and patterning of the metanephric nephron

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Abstract

The functional unit of the mammalian metanephric kidney is the nephron: a complex tubular structure dedicated to blood filtration and maintenance of several important physiological functions. Nephrons are assembled from a nephron-restricted pool of mesenchymal progenitors over an extensive developmental period that is completed prior to (human), or shortly after (mouse), birth. An appropriate balance in the expansion and commitment of nephron progenitors to nephron formation is essential for normal kidney function. Too few nephrons increases risk of kidney disease later in life while the failure of normal progenitor differentiation in Wilm's tumor patients leads to massive growth of a nephroblast population often necessitating surgical removal of the kidney. An inductive process within the metanephric mesenchyme leads to formation of a pretubular aggregate which transitions into an epithelial renal vesicle: the precursor for nephron assembly. Growth, morphogenesis and patterning transform this simple cyst-like structure into a highly elongated mature nephron with distinct cell types positioned along a proximal (glomerular) to distal (connecting segment) axis of functional organization. This review discusses our current understanding of the specification, maintenance and commitment of nephron progenitors, and the regulatory processes that transform the renal vesicle into a nephron.

Keywords

Nephron; nephrogenesis; induction; proximal/distal; patterning; Wnt signaling; Notch signaling

1. Introduction

The mammalian metanephric kidney is a highly complex organ that filters waste products from the circulation, maintains electrolyte and pH balance of body fluid, bone mineralization, blood pressure and blood composition. Many of these actions are carried out by the nephron: the repeated, functional unit of the kidney. The full complement of nephrons, on average 900,000 to 1 million in humans and 13,000 in the mouse, are assembled by early postnatal life [1,2]. The nephron serves as the blood-filtering component of the kidney. Filtration starts within renal corpuscle. Within the renal corpuscle, the tufted

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capillaries of the glomerulus are wrapped by interdigitating foot processes from the podocytes of the Bowman's capsule at the proximal end of the nephron. As blood pumps through the capillaries, the pressure pushes water and small solutes through a fenestrated endothelium, an intervening layer of basement membrane and finally between the foot processes. All three layers serve a role as sieves to maintain blood cells and essential proteins within the blood stream while allowing solutes such as salts, carbohydrates, and amino acids to pass through into the nephron. The filtrate is released into Bowman's space of the renal corpuscle where it subsequently enters the renal tubule for further modification [3]. The renal tubule consists of three major components: The proximal tubule, the loop of Henle, and the distal tubule each of which can be further subdivided by molecular and morphological criteria into subdomains. All three major segments have specialized roles in the physiological action of the kidney resorbing essential solutes and modifying the composition of the filtrate through distinct membrane channels and transporters present within different segments of the nephron [4]. This ensures essential components are returned to the blood stream, water, salt and pH homeostasis in tissue fluid is maintained, and waste products are excreted in the urine. Finally, the filtrate flows through a connecting segment into the collecting duct system and then via the ureter to the bladder where urine is stored prior to excretion.

As much of our understanding has developed around one experimental model system, the mouse, a general review of the developing mouse kidney follows to provide a framework for a more detailed mechanistic discussion of nephron induction and patterning.

2) Metanephric kidney and nephron progenitor specification

The urogenital system of mammals is derived from the intermediate mesoderm of the developing embryo. Paired, epithelial, nephric ducts arise dorsally from the intermediate mesoderm, and elongate caudally along left and right sides of the embryo. As the ducts grow, they induce waves of pronephric and mesonephric tubule formation from adjacent, mesenchymal intermediate mesoderm. These primitive tubular structures are transient and minimally functional, although a subset of mesonephric tubules contribute to structures of the male reproductive system [5].

The adult kidney, the metanephros, arises from intermediate mesoderm anlagen at the level of the hind-limb bud and is first evident at embryonic day 10.5 (E10.5). Here, a molecularly and developmentally distinct mesenchymal cell population, the metanephric mesenchyme, flanks the paired mesonephric ducts and contains progenitors of the future stromal and nephron components, as well as some of the vasculature [6–9]. This distinct population secretes signaling molecules including Gdnf and Fgf10 which engage with receptors of the nephric duct to induce outgrowth of a structure referred to as the ureteric bud [10]. As the ureteric bud grows towards the metanephric mesenchyme, the metanephric mesenchyme condenses around the bud tip with the various progenitor types occupying distinct positions within the condensate. The nephron progenitors which fate map to Six2+ and Cited1+ cells come to lie immediately adjacent to the ureteric epithelium while Foxd1+ cells that are the interstitial/stromal cell progenitors contact the outer surface of the Six2 domain [6–8,11,12]. A lengthy period of reciprocal signaling between these mesenchymal progenitor pools and

progenitors within the tip of the ureteric epithelium establishes a fully arborized collecting duct network and the species-specific nephron complement. In this process, signals initiating from the ureteric tip region maintain and expand uncommitted nephron progenitors and induce a subset of progenitors to initiate a program of nephrogenesis in conjunction with ureteric epithelial branching. Branching of the ureteric epithelium is regulated by signals from both interstitial and nephron progenitor cells [10,13–15]. Branching ceases and nephron progenitors are exhausted around postnatal day 3 (P3) with the formation of approximately ~3,400 nephron progenitor/tip niches and ~13,000 nephrons, although this number is dependent on the mouse strain [2,16–18]. Past this point, kidney growth is largely driven by proliferative expansion of the epithelial networks of the developing nephrons and collecting duct networks [14]. The molecular control of interstitial progenitor dynamics and differentiation is not well understood.

The first evidence of nephron progenitor commitment is the formation of a tightly packed pretubular aggregate beneath the branch tips that rapidly transitions to an epithelial renal vesicle. The renal vesicle undergoes growth and morphogenesis through comma- and S-shaped stages (Fig. 2) before fusing with the ureteric epithelium to establish a patent luminal connection between the nephron and urine transport network [19]. It is around the S-shaped body stage that the first evidence of vascular remodeling is evident in what is a vital but poorly understood process that interconnects vascular and nephron activities [9]. The stereotypical morphogenesis of the nephron anlagen is accompanied by a progressive elaboration of a proximal-distal axis of cellular complexity that ultimately gives rise to the mature nephron structure comprising from proximal to distal: parietal epithelium and podocytes of the Bowman's capsule at the vascular interface within the glomerulus, proximal tubule segments, loop of Henle, distal tubule, and a connecting segment fused to the collecting duct. Nephron structures are also orientated along a radial axis wherein the glomerulus, S1 and S2 segments of the proximal tubule, distal tubule and connecting segment are positioned within the outer cortex of the kidney, and the S3 segment of the proximal tubule and loop of Henle internally with the medullary region [20], (Fig. 2).

This review centers on the nephron and only considers the actions of other key cell types where they directly relate to nephrogenic programs. From the nephron perspective, we can distinguish several steps in generating a nephron: specification of the metanephric mesenchyme, maintenance and expansion of the nephron progenitor compartment, induction and commitment of nephron progenitors to the renal vesicle, morphogenesis and patterning of renal vesicle derivatives, and differentiation of mature cell types regulating nephron function. Several recent reviews provide broad insights into these processes [21–24]. Here, we focus on the regulatory controls governing commitment of nephron progenitors to the renal vesicle and subsequent patterning processes establishing proximal-distal cell fates in the developing nephron.

3) Commitment of nephron progenitors to renal vesicle formation

Sixty years ago Clifford Grobstein demonstrated that interactions between the ureteric bud and associated mesenchyme are essential for normal kidney development. Specifically, the mesenchyme failed to undergo differentiation in the absence of the ureteric bud. This

suggested that an inductive signal was missing and the ureteric bud was the source of this signal [25–27]. Additional studies by Saxen and colleagues established the prevailing view that nephrogenesis requires a permissive signal to enable the transition of mesenchymal progenitors to committed epithelial renal vesicles [28]. Genetic, biochemical and culture experiments argue that canonical Wnt signaling is the central pathway initiating mammalian nephrogenesis [29–32].

The mammalian Wnt family comprises nineteen, lipid-modified secreted glycoproteins several of which regulate distinct aspects of mammalian kidney development [29,33–38]. Wnt9b is expressed uniformly in the epithelium of the ureteric bud on outgrowth, but is progressively down-regulated in epithelial progenitors within the ureteric tip adjacent to the nephrogenic mesenchyme, and maintained at higher levels beneath the tip where pretubular aggregates first emerge; elevated levels are maintained throughout much of the cortical and outer medullary collecting duct network. Genetic mutation has demonstrated that Wnt9b is not required for specification of nephron progenitors, or outgrowth of the ureteric bud, but is essential for renal vesicle induction. Further, Wnt9b producing cells can induce renal vesicle formation in isolated metanephric mesenchyme [29]. Genetic evidence suggests that Wnt9b inductive properties are mediated through the stabilization of β -catenin, a critical regulatory event in canonical Wnt signaling. In this, accumulation of cytoplasmic β -catenin enables nuclear translocation and dimerization with Lef/Tcf-HMG box containing DNA-binding factors, switching Lef/Tcf complexes from transcriptional repressors to transcriptional activators [29]. Wnt9b inductive activity can be substituted by genetic stabilization of β -catenin within Six2+ nephron progenitors, providing strong evidence that Wnt9b acts, at least in part, through a canonical-like signaling mechanism [30,31].

Wnt9b signaling activates expression of secondary signals, Fgf8 and a second Wnt member, Wnt4, within pretubular aggregates [29,39] (Fig. 1). Both signals are required for normal development of renal vesicles downstream of Wnt9b [34,35,40,41]. Genetic analysis suggests Fgf8 is required for Wnt4 activation, and Wnt4 signaling is required to maintain both Fgf8 expression and its own activity, and *de novo* activation of the transcriptional regulator Lhx1 [40,41]. The observation that stabilized β -catenin can rescue Lhx1 activation argues for a continued role of a canonical-like action downstream of Wnt4 [31]. Direct evidence that β -catenin bound Lef/Tcf complexes regulate Fgf8 and Wnt4 transcription comes from chromatin immunoprecipitation (ChIP) experiments using small molecules to stabilize β -catenin in Six2+ progenitors mimicking ligand induced activation, then antibodies directed against β -catenin to pull-down β -catenin complexes engaged at DNA targets. β -catenin ChIP-seq identified direct interactions with putative regulatory regions flanking Fgf8 and Wnt4 and transgenic analysis confirmed Lef/Tcf-binding site dependent renal vesicle enhancer activity for regulatory regions at predicted enhancers for Wnt4 and Fgf8 [32].

While there is considerable evidence consistent with a direct action of Lef/Tcf/ β -catenin complexes downstream of both Wnt9b and Wnt4, genetic stabilization of β -catenin blocks transition of induced metanephric mesenchyme to renal vesicles, and consequently progression of the nephrogenic program. Thus, termination of canonical signaling is likely

important for completion of the mesenchymal to epithelial transition, and Wnt4 action may switch from a canonical to non-canonical signaling pathway in this process [30,32,42].

Cell lineage and cell fate analysis at E10.5 indicate that Six2⁺ progenitors comprise a self-renewing, multipotent progenitor population for the entire period of nephrogenesis [7]. Clearly, only a fraction of cells commits to active nephrogenesis at each round of ureteric branching raising the question of how canonical Wnt signaling is regulated to restrict induction and enable expansion of the nephron progenitor pool. Interestingly, in the absence of Six2 all nephron progenitors undergo a rapid, premature differentiation forming ectopic renal vesicles providing strong evidence that Six2, a transcriptional regulatory factor, blocks renal vesicle differentiation within nephron progenitors [12]. As Six2 and Wnt9b compound mutants resemble Wnt9b single mutants in that they lack renal vesicles, these genetic studies suggest Six2 activity counters Wnt9b's nephron inducing action [7]. How Six2 acts is not clear but the demonstrations that Six2 can complex with Lef/Tcf factors and is bound at the Fgf8 and Wnt4 enhancers engaged by β -catenin suggests that Six2 may play a direct role in suppressing the activation of these targets [31].

While the genetic evidence suggests that the bulk nephron progenitor population requires Six2 to maintain progenitor status and to remain refractory to Wnt9b signaling, a subset of Six2⁺ cells is induced at each round of branching [7,12]. Further, Six2 levels remain high within induced nephron progenitors, and even persist into renal vesicle stage [32,39], suggesting that Six2 alone cannot block the inductive process in all nephron progenitors, and there are likely additional regulatory inputs into renal vesicle induction. The action of Wnt9b signaling on Six2⁺ nephron progenitors is also more complex. Genetic and biochemical evidence points to a positive role for Wnt9b in maintaining this progenitor population through direct transcription regulation of progenitor target genes [43], (Fig. 1).

Recent studies argue that one additional input comes from the Foxd1⁺ interstitial progenitors that surround Six2⁺ nephron progenitors. Ablation of this population leads to an expansion of the nephron progenitors and a marked delay in the inductive process suggesting that the lack of stromal signals may block induction of the nephrogenic program [44]. Further analysis revealed a role for stromal Fat4 and the Hippo/Warts pathway in modulating β -catenin activity. Uninduced progenitors retain nuclear Taz/Yap, and deletion of these components within the nephron progenitor population significantly reduces the number of differentiated structures. Additionally, despite retaining Six2⁺ progenitors, the expression of progenitor specific β -catenin targets such as Cited1 is severely affected [43,44,45]. These data suggest a model wherein nuclear Taz/Yap cooperates with β -catenin to promote expression of progenitor-associated genes. However, when nephron progenitors encounter Fat4 on the surface of neighboring stromal cells Taz/Yap is exported from the nucleus enabling the expression of Wnt9b target genes Fgf8 and Wnt4, thereby linking stromal signals to progenitor induction [44], (Fig. 1). The mechanistic detail of the crosstalk between Hippo and Wnt signaling pathways in nephron progenitors remains to be determined.

Bmp7 is a signaling factor produced by several cell types in the kidney, including the nephron progenitors. Knockout studies reveal massive apoptosis of the mesenchyme in the

absence of Bmp7, suggesting an integral role in maintaining the nephron progenitor population [46–50]. In addition, deletion of Bmp7 after E12.5 induces precocious differentiation of nephron progenitors [51]. Recent studies suggest Bmp7 activity is also important for progenitor differentiation [52,53]. As discussed earlier, Six2 expression persists beyond the progenitor population into newly induced structures while Cited1, a transcriptional component with no demonstrated kidney function [54], more specifically marks uncommitted nephron progenitors. Culture studies show that Cited1+ cells are refractory to induction, and the transition to a Six2+Cited1- state is mediated by Bmp7-SMAD signaling. At this point, cells do not express markers indicative of Wnt induction such as the Wnt-target and Wnt transcriptional regulator Lef1, but rather they are primed for induction by Wnt mediated signals (Fig. 1). These *in vitro* data correlate with expression domains of Six2, Cited1, and Lef1 *in vivo*, and with Bmp7 mutants which have reduced numbers of the Six2+Cited1-Lef1- population [52].

Additional, albeit indirect, support for a role of Bmp7 in the inductive process *in vivo* comes from analysis of Foxd1 mutants. Foxd1 mutants exhibit an expanded domain of nephron progenitors reflecting a failure of progenitor transition to a nephrogenic program. In the absence of Foxd1, cortical interstitial progenitors produce high levels of the extracellular proteoglycan decorin (Dcn) that is normally expressed only at high levels by differentiating interstitium in medullary regions, and at lower levels within the cortical domain. *In vitro* studies show that Dcn antagonizes Bmp7 activity. Consistent with this action, reduced numbers of pSMAD1/5+ nephron progenitors are present in the mutants. Compound mutants of Foxd1 and Dcn can partially rescue the phenotype elevating the number of cells expressing differentiation markers and pSMAD1/5. These data suggest that Dcn expression in Foxd1 mutants inhibits Bmp7-SMAD signaling leading to the failure of nephron progenitor induction. Therefore, the role of Foxd1 includes repressing the expression of genes that promote later stromal differentiation, thereby allowing the normal progression of nephron progenitor differentiation that is linked to Bmp7-SMAD signaling [53], (Fig. 1). How these dual roles for Bmp7 in maintenance and induction are differentially regulated is unknown. Interestingly, the requirement for Bmp7 in nephron induction is not necessary at early stages of development as E11.5 progenitors can undergo direct induction without Bmp7 priming [52]. The nephron progenitor maintenance role may be predominant at early stages when Bmp7 is not yet required for induction

Nephron induction is a developmentally limited process; Six2+ nephron progenitors are lost by P2-3 in the mouse, and around 36 weeks of gestation in man [16,17,55]. Once lost, the capacity for *de novo* nephrogenesis ceases, and repair of existing nephrons is the only normal reparative strategy for a damaged kidney [56]. Why the self-renewal ceases and coordinated wave of differentiation occurs remains to be determined.

Analysis of gene expression changes in the postnatal kidney shows that loss of expression of nephron progenitor markers such as Cited1 and Six2 precedes that of ureteric tip markers [16,17]. Even though the nephron progenitors are lost, ureteric epithelium from P3 kidneys is competent to branch and support nephron progenitor survival and differentiation [16]. These data suggest that loss of the nephron progenitor population is the driving force behind cessation. While the proliferation rates of both the nephron progenitors and the ureteric tip

progenitors decreases across developmental time, the ratio of nephron progenitor cells to tip cells also decreases, suggesting a steady depletion of nephron progenitors leading up to cessation [18]. Additionally, markers of differentiation such as Wnt4 appear within the remaining P2 nephron progenitors, and Six2 expression persists into differentiating nephron structures. Together these observations suggest Six2 expression is not sufficient to retain the progenitors in refractory state. Wnt9b expression extends into the tips postnatally and may overlap with markers of tip identity such as Wnt11, perhaps overriding the balance of signal required to support nephron progenitor proliferation and instead setting off the inductive response [17]. In addition to the expression of differentiation markers within the progenitors, RNA-seq analysis of P0 versus P2 nephron progenitors shows a decrease in the expression of glycolytic pathway genes at P2 [57]. The increase of oxygen tension at birth may also trigger the coordinated and rapid differentiation through metabolic changes within the progenitors though clearly this cannot account for the cessation of human nephrogenesis prior to parturition [55]. Despite the depletion of this essential population of cells, the final number of nephrons formed is more than sufficient for survival as proven by the wide range of nephron numbers found in the human population, although low number is likely a predisposing factor for kidney disease later in life [58, 59].

4) Proximal-distal patterning of renal vesicle derivatives

Lineage tracing of nephron progenitors shows clonal descendants give rise to several distinct regions of the forming nephron [6,7]. Consequently, the forming renal vesicle is thought to comprise an assembly of uncommitted cell types. But when and how does this ball of cells acquire polarity and initiate patterning processes that lead to formation of the mature cell types of the nephron?

The first indications of polarity are early: a proximal/distal polarity is evident in the polarized expression of several genes at renal vesicle and even pretubular aggregate stages [39, 60–62]. How this polarity is initiated is unclear but continued interactions with the ureteric epithelium could play a role as genes that occupy distal domains in the forming nephron are active in regions of the renal vesicle that underlie the ureteric epithelium [62]. Thereafter, intrinsic interactions within polarized renal vesicles are likely to play the major role in ongoing patterning processes. Wnt4 and Lef1 show polarized expression patterns in the distal renal vesicle suggesting a Wnt signaling gradient may be one contributory factor to the regulation of early renal vesicle polarity [39]. But the best evidence underscores a critical role for polarized Notch signaling in the induction of proximal nephron fates (Fig. 2).

Several, Notch pathway components display early polarized expression patterns consistent with a role for Notch signaling nephron patterning [62,63]. Loss of Notch signaling through modulation of general pathway components such as γ -secretase or presenilins results in nephron structures that lack proximal (podocyte and proximal tubule) fates [64,65]. To more specifically address Notch actions in nephrogenesis, a metanephric mesenchyme specific knockout of Notch2 was analyzed: Notch2 deficient kidneys initiate segmentation but only form distal epithelial structures demonstrating a requirement for Notch2 in proximal patterning [66]. Although Notch1 expression overlaps with Notch2 and the Notch

intracellular domain 1 (NICD1) can compensate for Notch2 loss, more efficient cleavage of Notch2 ensures Notch2 predominates at the cell surface [66,67]. Three Notch ligands – Dll1, Lfng and Jag1 – are well placed within distal (early renal vesicle) or medial (S-shaped body stage) regions of the developing nephron to regulate proximal Notch signaling [62,68–70]. Consistent with a role for Dll, Dll hypomorphs have reduced nephron numbers and lack proximal tubule segments [66].

During development, Notch signaling operates downstream of Wnt-mediated induction of renal vesicle progenitors which begs the question of whether prior Wnt input is needed for Notch action? This does not appear to be the case. Ectopic Notch activation in Six2+ nephron progenitors is sufficient to trigger a mesenchymal to epithelial transition in the absence of Wnt9b and Wnt4, and the induction of proximal (podocyte and proximal tubule) cell fates [71,72]. Thus, it is likely that the primary role of the early inductive pathway is to establish the appropriate epithelial context to restrict and shape the signaling interactions that are critical for subsequent patterning of the nephron.

How does Notch signaling differentially contribute to the establishment of podocyte vs proximal tubule identity? Answers to this question may involve the differential expression of other factors to further refine downstream programs. Wt1 is a transcription factor expressed in the proximal renal vesicle and more specifically restricted to the developing podocytes where it is required for their maturation [62,73,74]. The Notch transcriptional mediator Rbpj can directly complex with Wt1 and Foxc2, another essential podocyte factor, and regulate expression *in vitro* of genes necessary for podocyte development [75,76]. These data suggest that while Notch signaling dictates proximal (podocyte and tubular) identity, Wt1 refines the program for podocyte development (Fig. 2). Wt1 also modulates the activity of Pax2 in the developing nephron. Pax2 is a transcription factor essential for normal kidney development [77,78]. Pax2 is expressed within nephron progenitors and early differentiating structures but its expression is downregulated in the presumptive podocyte progenitors as Wt1 expression increases. These data suggest Wt1 may antagonize Pax2 expression. Consistent with this view, Wt1 binding sites were identified upstream of the Pax2 transcriptional start site, in a region that shows Wt1-mediated repression in cell culture reporter assays. Therefore, it is likely that Wt1 promotes podocyte development through direct repression of proximal Pax2 activity [73], (Fig. 2).

Additional links to Notch signaling and nephron patterning have arisen through studies of Hnf1b, a POU domain containing transcription factor (Fig. 2). Removal of Hnf1b in nephron progenitors or pretubular aggregates leads to a striking phenotype: dilated glomeruli that attach to the collecting duct by a short tubule: the proximal tubule and loop of Henle are completely absent. A partially overlapping phenotype with Notch2 mutants could reflect a deficiency in normal Notch signaling. Consistent with this view, expression of the Notch ligands Dll1, Lfng and Jag1 are reduced with loss of Hnf1b [70,79]. Further, Hnf1b is bound at the promoter of Dll1 and Lfng suggesting direct regulation. Hnf1b also associates with the promoters of Irx1 and Irx2 [70], transcriptional regulators expressed within the medial segment of the S-shaped body that are down-regulated in differentiating structures of Hnf1b mutants [70,79]. These data suggest a possible role for Irx1/2 downstream of Hnf1b,

although no direct role in the patterning process has been demonstrated for either of these factors.

As opposed to the role of Notch signaling in proximal identity, the control of medial and distal identity is highlighted by the actions of *Brn1* and possibly *Lgr5* (Fig. 2). *Lhx1* expression demarcates the distal RV, together with *Dll1*, and a transcription factor *Brn1* [62,63,80]. Metanephric mesenchyme specific removal of *Lhx1* results in renal vesicles that lack these markers of distal polarity, although distal expression of *Wnt4* is retained. Normal S-shaped bodies are absent in *Lhx1* mutants [63]. Direct *Brn1* mutants develop kidneys with normal glomerular and proximal tubule counts, however, elongation and differentiation of the loop of Henle is severely reduced as is differentiation of the distal tubule including the macula densa, specialized salt-sensing cells located in this segment. Together these results suggest an early role for *Brn1* is regulating medial and distal identity within the S-shaped body [80]. *Lgr5* encodes a surface receptor that marks several Wnt responsive stem cell niches in the adult, including the intestine and hair follicle [81]. However, in the mammalian kidney *Lgr5* is not expressed in early progenitor populations but is activated in the distal segment of the comma- and S-shaped body. Fate mapping studies show these cells give rise to the thick ascending loop of Henle and the distal convoluted tubule. Unlike other organs, the expression of *Lgr5* is limited to the developing kidney; *Lgr5* expression is lost in mature nephrons and is not found in the adult kidney though injury-repair models have not been investigated [82]. The current data suggest that if *Lgr5* has a function in the kidney, it is likely restricted to medial and/or distal tubule development.

While there is continuous progress in the identification of genes involved in the establishment of nephron polarity and the broader context of proximal and distal identity, little is known about how terminal differentiation of the various segments is accomplished. Clearly this is a complex problem and requires a further consideration of nephron function which highlights the specific actions of regional cell types in renal physiology. Understanding terminally differentiated states and the underpinning molecular mechanisms will tie developmental programs to physiological actions in the mature organ system.

5) Conclusions and perspective

The induction and patterning of the nephron is a complex process that takes a single progenitor population and forms a highly specialized structure capable of carrying out distinct and complex physiological actions. While the field continues to advance on several fronts, there is still a large gap in our knowledge of nephrogenesis. A broad picture of the molecular control of nephron progenitors, their induction, commitment to nephrogenesis, and subsequent nephron patterning has emerged but there are likely many important players that remain to be discovered, particularly in late stage regulation of differentiated cell states. More systematic approaches should accelerate discovery and understanding here. RNA-seq and ChIP-seq applied to the nephron progenitor population has provided new insights into progenitor programs at a different scale than previous technologies [32,57,83]. The Genitourinary Developmental Molecular Anatomy Project (GUDMAP; www.gudmap.org) sponsored by the National Institute for Diabetes, Digestive and Kidney Disease has developed an impressive database of gene expression data that transforms the histological

view of the kidney that prevailed 10 years ago to discrete, annotated molecularly defined cell populations consistent with a 21st century view of organ anatomy. Further, GUDMAPs transgenic resource has created new mouse strains to visualize, isolate and genetically modify key cell populations within the kidney, and other organs comprising the urogenital system [84].

Most of our current understanding has come from *in vivo* genetic approaches that while insightful are also time consuming, technically demanding and expensive. Further, as reflected by this review we know a considerable amount about one mammalian model but very little about others, most notably our own kidney development. There is clearly a vast scope for improvement of the limited *in vitro* models to fully translate our knowledge of nephrogenic programs and to bring human kidney development to research scrutiny for clinical advancement. Recent advances in manipulating mouse and human stem cells are encouraging in this regard. Several recent studies have utilized embryo stem cells or induced pluripotent stem cells to generate cell types on the developmental pathway to the kidney [85] or more recently of the kidney itself [86–88]. Importantly, *in vitro* derived human kidney cell types have been shown to incorporate *in vitro* into ureteric bud structures, differentiate into tubule and glomerulus like structures, the latter of which was capable of blood vessel recruitment *in vivo*, or self-assemble and differentiate *in vitro* into nephron-like structures when aggregated with mouse kidney explants [86–88]. These studies demonstrated not only form but also function, and are a critical first step towards the development of new therapeutic strategies for the treatment of kidney injury and disease.

To date, no *bona fide* multi-potent stem cell of the adult kidney has been identified. Once nephrogenesis is complete, the number of nephrons is set; no new nephrons form thereafter, even in a severely injured kidney. Terminally differentiated cells of the nephron have been thought to turn over slowly in the adult kidney as a plausible mechanism for cell renewal. This view has recently been challenged through clonal labeling approaches that suggest higher day-to-day turnover of tubule cells and large clonal contributions to the nephron suggesting rare, segment specific progenitors rather than any differentiated cell-type is a key mode for renewal of tubule epithelium [89]. Further, there is evidence for podocyte replacement by cells of the parietal epithelium of the Bowman's capsule that share a Wt1 lineage relationship with immediately adjacent podocytes [90,91]. Acute kidney injury can result in the loss of as many as 80% of cells in the most sensitive regions of the proximal tubule. Here, the balance of evidence in the mouse, where definitive experiments are possible, suggests a mechanism of intrinsic cellular regeneration fueled by surviving proximal tubule cells [56,92,93] or by enhanced expansion of segment specific progenitor types [89]. No compelling evidence suggest a multipotent stem-based mechanism of repair. However, arguments have been raised for a rare nephron stem cell in human that can contribute to kidney repair [94,95]. A common view holds that kidney repair recapitulates developmental processes. While several nephrogenic genes are reported to be re-expressed after injury, there is no substantial evidence to support a simple developmental recapitulation model [96,97]. Additional clarification of the cell types and mechanisms at play in restoring renal function in the mouse will enable a more rigorous scrutiny of these processes in the human kidney.

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References

- Bertram JF, Douglas-Denton RN, Diouf B, Hughson MC, Hoy WE. Human nephron number: implications for health and disease. *Ped Neph.* 2011; 26:1529–33.
- Cullen-McEwen LA, Kett MM, Dowling J, Anderson WP, Bertram JF. Nephron number, renal function, and arterial pressure in aged GDNF heterozygous mice. *Hypertension.* 2003; 41:335–40. [PubMed: 12574104]
- Quaggin SE, Kreidberg JA. Development of the renal glomerulus: good neighbors and good fences. *Development.* 2008; 135:609–20. [PubMed: 18184729]
- Thiagarajan RD, Georgas KM, Rumballe BA, Lesieur E, Chiu HS, Taylor D, Tang DT, Grimmond SM, Little MH. Identification of anchor genes during kidney development defines ontological relationships, molecular subcompartments and regulatory pathways. *PLoS One.* 2011; 6(2):e17286. [PubMed: 21386911]
- Davidson, AJ. StemBook. Mouse kidney development. The Stem Cell Research Community, StemBook; 2009.
- Boyle S, Misfeldt A, Chandler KJ, Deal KK, Southard-Smith EM, Mortlock DP, Baldwin HS, de Caestecker M. Fate mapping using Cited1-CreERT2 mice demonstrates that the cap mesenchyme contains self-renewing progenitor cells and gives rise exclusively to nephronic epithelia. *Dev Biol.* 2008; 313:234–45. [PubMed: 18061157]
- Kobayashi A, Valerius MT, Mugford JW, Carroll TJ, Self M, Oliver G, McMahon AP. Six2 defines and regulates a multipotent self-renewing nephron progenitor population throughout mammalian kidney development. *Cell Stem Cell.* 2008; 3:169–81. [PubMed: 18682239]
- Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, et al. Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. *Am J of Pathol.* 2010; 176:85–97. [PubMed: 20008127]
- Sequeira Lopez ML, Gomez RA. Development of the renal arterioles. *J Am Soc Nephrol.* 2011; 22:2156–65. [PubMed: 22052047]
- Costantini F. Genetic controls and cellular behaviors in branching morphogenesis of the renal collecting system. *Dev Biol.* 2012; 1:693–713.
- Hatini V, Huh SO, Herzlinger D, Soares VC, Lai E. Essential role of stromal mesenchyme in kidney morphogenesis revealed by targeted disruption of Winged Helix transcription factor BF-2. *Genes Dev.* 1996; 10:1467–78. [PubMed: 8666231]
- Self M, Lagutin OV, Bowling B, Hendrix J, Cai Y, Dressler RD, et al. Six2 is required for suppression of nephrogenesis and progenitor renewal in the developing kidney. *EMBO J.* 2006; 25:5214–28. [PubMed: 17036046]
- Costantini F, Kopan R. Patterning a complex organ: Branching morphogenesis and nephron segmentation in kidney development. *Dev Cell.* 2010; 18:698–712. [PubMed: 20493806]
- Mendelsohn C, Batourina E, Fung S, Gilbert T, Dodd J. Stromal cells mediate retinoid-dependent functions essential for renal development. *Development.* 1999; 126:1139–48. [PubMed: 10021334]
- Rosselot C, Spraggon L, Chia I, Batourina E, Riccio P, Lu B, et al. Non-cell-autonomous retinoid signaling is crucial for renal development. *Development.* 2010; 137:283–92. [PubMed: 20040494]
- Hartman HA, Lai HL, Patterson LT. Cessation of renal morphogenesis in mice. *Dev Biol.* 2007; 310:379–87. [PubMed: 17826763]
- Rumballe BA, Georgas KM, Combes AN, Ju AL, Gilbert T, Little MH. Nephron formation adopts a novel spatial topology at cessation of nephrogenesis. *Dev Biol.* 2011; 360:110–22. [PubMed: 21963425]

18. Short KM, Combes AN, Lefevre J, Ju AL, Georgas KM, Lamberton T, et al. Global quantification of tissue dynamics in the developing mouse kidney. *Dev Cell*. 2014; 29:188–202. [PubMed: 24780737]
19. Kao RM, Vasilyev A, Miyawaki A, Drummond IA, McMahon AP. Invasion of distal nephron precursors associates with tubular interconnection during nephrogenesis. *J Am Soc Nephrol*. 2012; 23:1682–90. [PubMed: 22904347]
20. Jacobson HR. Functional segmentation of the mammalian nephron. *Am J Physiol Renal Physiol*. 1981; 241:F203–218.
21. Little MH, McMahon AP. Mammalian kidney development: principles, progress, and projections. *Cold Spring Harb Perspect Biol*. 2012; 4:a008300. [PubMed: 22550230]
22. Carroll TJ, Das A. Defining the signals that constitute the nephron progenitor niche. *J Am Soc Nephrol*. 2013; 24:873–6. [PubMed: 23578945]
23. Kopan R, Chen S, Little M. Nephron Progenitor Cells: Shifting the Balance of Self-Renewal and Differentiation. *Curr Top Dev Biol*. 2014; 107:293–331. [PubMed: 24439811]
24. Pietilä I, Vainio SJ. Kidney development: an overview. *Nephron Exp Nephrol*. 2014; 126:40. [PubMed: 24854638]
25. Grobstein C. Inductive epithelio-mesenchymal interaction in cultured organ rudiments of the mouse metanephros. *Science*. 1953; 118:52–55. [PubMed: 13076182]
26. Grobstein C. Inductive interaction in the development of the mouse metanephros. *J Exp Zool*. 1955; 130:319–340.
27. Grobstein C. Trans-filter induction of tubules in mouse metanephrogenic mesenchyme. *Exp Cell Res*. 1956; 10:424–440. [PubMed: 13317909]
28. Saxén L, Sariola H. Early organogenesis of the kidney. *Pediatr Nephrol*. 1987; 1:385–92. [PubMed: 3153305]
29. Carroll TJ, Park JS, Hayashi S, Majumdar A, McMahon AP. Wnt9b plays a central role in the regulation of mesenchymal to epithelial transitions underlying organogenesis of the mammalian urogenital system. *Dev Cell*. 2005; 9:283–92. [PubMed: 16054034]
30. Kuure S, Popsueva A, Jakobson M, Sainio K, Sariola H. Glycogen synthase kinase-3 inactivation and stabilization of b-catenin induce nephron differentiation in isolated mouse and rat kidney mesenchymes. *J Am Soc Nephrol*. 2007; 18:1130–39. [PubMed: 17329570]
31. Park JS, Valerius MT, McMahon AP. Wnt/ β -catenin signaling regulates nephron induction during mouse kidney development. *Development*. 2007; 134:2533–39. [PubMed: 17537789]
32. Park JS, Ma W, O'Brien LL, Chung E, Guo JJ, Cheng JG, et al. Six2 and Wnt regulate self-renewal and commitment of nephron progenitors through shared gene regulatory networks. *Dev Cell*. 2012; 23:637–51. [PubMed: 22902740]
33. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol*. 2004; 20:781–810. [PubMed: 15473860]
34. Stark K, Vainio S, Vassileva G, McMahon AP. Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4. *Nature*. 1994; 372:679–683. [PubMed: 7990960]
35. Kispert A, Vainio S, McMahon AP. Wnt-4 is a mesenchymal signal for epithelial transformation of metanephric mesenchyme in the developing kidney. *Development*. 1998; 125:4225–34. [PubMed: 9753677]
36. Majumdar A, Vainio S, Kispert A, McMahon J, McMahon AP. Wnt11 and Ret/Gdnf pathways cooperate in regulating ureteric branching during metanephric kidney development. *Development*. 2003; 130:3175–85. [PubMed: 12783789]
37. Yu J, Carroll TJ, Rajagopal J, Kobayashi A, Ren Q, McMahon AP. A Wnt7b-dependent pathway regulates the orientation of epithelial cell division and establishes the corticomedullary axis of the mammalian kidney. *Development*. 2009; 136:161–71. [PubMed: 19060336]
38. Karner CM, Chirumamilla R, Aoki S, Igarashi P, Wallingford JB, Carroll TJ. Wnt9b signaling regulates planar cell polarity and kidney tubule morphogenesis. *Nat Genet*. 2009; 41:793–9. [PubMed: 19543268]

39. Mugford JW, Yu J, Kobayashi A, McMahon AP. High-resolution gene expression analysis of the developing mouse kidney defines novel cellular compartments within the nephron progenitor population. *Dev Biol.* 2009; 333:312–23.10.1016/j.ydbio.2009.06.043 [PubMed: 19591821]
40. Perantoni AO, Timofeeva O, Naillat F, Richman C, Pajni-Underwood S, Wilson C, Vainio S, Dove LF, Lewandoski M. Inactivation of FGF8 in early mesoderm reveals an essential role in kidney development. *Development.* 2005; 132:3859–71. [PubMed: 16049111]
41. Grieshammer U, Cebrian C, Ilagan R, Meyers E, Herzlinger D, Martin GR. FGF8 is required for cell survival at distinct stages of nephrogenesis and for regulation of gene expression in nascent nephrons. *Development.* 2005; 132:3847–57. [PubMed: 16049112]
42. Tanigawa S, Wang H, Yang Y, Sharma N, Tarasova N, Ajima R, Yamaguchi TP, Rodriguez LG, Perantoni AO. Wnt4 induces nephronic tubules in metanephric mesenchyme by a non-canonical mechanism. *Dev Biol.* 2011; 352:58–69. [PubMed: 21256838]
43. Karner CM, Das A, Ma Z, Self M, Chen C, Lum L, Oliver G, Carroll TJ. Canonical Wnt9b signaling balances progenitor cell expansion and differentiation during kidney development. *Development.* 2011; 138:1247–57. [PubMed: 21350016]
44. Das A, Tanigawa S, Karner CM, Xin M, Lum L, Chen C, Olson EN, Perantoni AO, Carroll TJ. Stromal-epithelial crosstalk regulates kidney progenitor cell differentiation. *Nat Cell Biol.* 2013; 15:1035–44. [PubMed: 23974041]
45. Reginensi A, Scott RP, Gregorieff A, Bagherie-Lachidan M, Chung C, Lim DS, et al. Yap- and Cdc42-dependent nephrogenesis and morphogenesis during mouse kidney development. *PLoS Genet.* 2013; 9:e1003380. [PubMed: 23555292]
46. Lyons KM, Hogan BL, Robertson EJ. Colocalization of BMP 7 and BMP 2 RNAs suggests that these factors cooperatively mediate tissue interactions during murine development. *Mech Dev.* 1995; 50:71–83. [PubMed: 7605753]
47. Dudley AT, Lyons KM, Robertson EJ. A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. *Genes Dev.* 1995; 9:2795–2807. [PubMed: 7590254]
48. Luo G, Hofmann C, Bronckers AL, Sohocki M, Bradley A, et al. BMP-7 is an inducer of nephrogenesis, and is also required for eye development and skeletal patterning. *Genes Dev.* 1995; 9:2808–20. [PubMed: 7590255]
49. Dudley AT, Robertson EJ. Overlapping expression domains of bone morphogenetic protein family members potentially account for limited tissue defects in BMP7 deficient embryos. *Dev Dyn.* 1997; 208:349–62. [PubMed: 9056639]
50. Dudley AT, Godin RE, Robertson EJ. Interaction between FGF and BMP signaling pathways regulates development of metanephric mesenchyme. *Genes Dev.* 1999; 13:1601–13. [PubMed: 10385628]
51. Tomita M, Asada M, Asada N, Nakamura J, Oguchi A, Higashi AY, et al. Bmp7 maintains undifferentiated kidney progenitor population and determines nephron numbers at birth. *PLoS One.* 2013; 8:e73554. [PubMed: 23991197]
52. Brown AC, Muthukrishnan SD, Guay JA, Adams DC, Schafer DA, Fetting JL, et al. Role for compartmentalization in nephron progenitor differentiation. *Proc Natl Acad Sci.* 2013; 110:4640–45. [PubMed: 23487745]
53. Fetting JL, Guay JA, Karolak MJ, Iozzo RV, Adams DC, Maridas DE, et al. FOXD1 promotes nephron progenitor differentiation by repressing decorin in the embryonic kidney. *Development.* 2014; 141:17–27. [PubMed: 24284212]
54. Boyle S, Shioda T, Perantoni AO, de Caestecker M. Cited1 and Cited2 are differentially expressed in the developing kidney but are not required for nephrogenesis. *Dev Dyn.* 2007; 236:2321–30. [PubMed: 17615577]
55. Hinchliffe SA, Sargent PH, Howard CV, Chan YF, van Velzen D. Human intrauterine renal growth expressed in absolute number of glomeruli assessed by the disector method and Cavalieri principle. *Lab Inve.* 1991; 64:777–84.
56. Humphreys BD, Valerius MT, Kobayashi A, Mugford JW, Soeung S, Duffield JS, et al. Intrinsic epithelial cells repair the kidney after injury. *Cell Stem Cell.* 2008; 2:284–91. [PubMed: 18371453]

57. Brunskill EW, Lai HL, Jamison DC, Potter SS, Patterson LT. Microarrays and RNA-Seq identify molecular mechanisms driving the end of nephron production. *BMC Dev Biol.* 2001; 11:15. [PubMed: 21396121]
58. Nyengaard JR, Bendtsen TF. Glomerular number and size in relation to age, kidney weight and body surface in normal man. *Anat Rec.* 1992; 232:194–201. [PubMed: 1546799]
59. Hoy WE, Bertram JF, Denton RD, Zimanyi M, Samuel T, Hughson MD. Nephron number, glomerular volume, renal disease and hypertension. *Curr Opin Nephrol Hypertens.* 2008; 17:258–65. [PubMed: 18408476]
60. Georgas K, Rumballe B, Wilkinson L, Chiu HS, Lesieur E, Gilbert T, et al. Use of dual section mRNA in situ hybridisation/immunohistochemistry to clarify gene expression patterns during the early stages of nephron development in the embryo and in the mature nephron of the adult mouse kidney. *Histochem Cell Biol.* 2008; 130:927–42. [PubMed: 18618131]
61. Brunskill EW, Aronow BJ, Georgas K, Rumballe B, Valerius MT, Aronow J, et al. Atlas of gene expression in the developing kidney at microanatomic resolution. *Dev Cell.* 2008; 15:781–91. [PubMed: 19000842]
62. Georgas K, Rumballe B, Valerius MT, Chiu HS, Thiagarajan RD, Lesieur E, et al. Analysis of early nephron patterning reveals a role for distal RV proliferation in fusion to the ureteric tip via a cap mesenchyme-derived connecting segment. *Dev Biol.* 2009; 332:273–86. [PubMed: 19501082]
63. Kobayashi A, Kwan KM, Carroll TJ, McMahon AP, Mendelsohn CL, Behringer RR. Distinct and sequential tissue-specific activities of the LIM-class homeobox gene *Lim1* for tubular morphogenesis during kidney development. *Development.* 2005; 132:2809–23. [PubMed: 15930111]
64. Cheng HT, Miner J, Lin M, Tansey MG, Roth KA, Kopan R. g-secretase activity is dispensable for the mesenchyme-to-epithelium transition but required for proximal tubule formation in developing mouse kidney. *Development.* 2003; 130:5031–41. [PubMed: 12952904]
65. Wang P, Pereira FA, Beasley D, Zheng H. Presenilins are required for the formation of comma- and S-shaped bodies during nephrogenesis. *Development.* 2003; 130:5019–29. [PubMed: 12930775]
66. Cheng HT, Kim M, Valerius MT, Surendran K, Schuster-Gossler K, Gossler A, et al. Notch2, but not Notch1, is required for proximal fate acquisition in the mammalian nephron. *Development.* 2007; 134:801–11. [PubMed: 17229764]
67. Liu Z, Chen S, Boyle S, Zhu Y, Zhang A, Piwnicka-Worms DR, et al. The extracellular domain of Notch2 increases its cell-surface abundance and ligand responsiveness during kidney development. *Dev Cell.* 2013; 25:585–98. [PubMed: 23806616]
68. Piscione TD, Wu MY, Quaggin SE. Expression of hairy/enhancer of split genes, *Hes1* and *Hes5*, during murine nephron morphogenesis. *Gene Expr Patterns.* 2004; 4:707–11. [PubMed: 15465493]
69. Chen L, Al-Awqati Q. Segmental expression of Notch and Hairy genes in nephrogenesis. *Am J Physiol Renal Physiol.* 2005; 288:F939–F952. [PubMed: 15821257]
70. Heliot C, Desgrange A, Buisson I, Prunskaitė-Hyyryläinen R, Shan J, Vainio S, et al. HNF1B controls proximal-intermediate nephron segment identity in vertebrates by regulating Notch signaling components and *Irx1/2*. *Development.* 2013; 140:873–85. [PubMed: 23362348]
71. Fujimura S, Jiang Q, Kobayashi C, Nishinakamura R. Notch2 activation in the embryonic kidney depletes nephron progenitors. *J Am Soc Nephrol.* 2010; 21:803–10. [PubMed: 20299358]
72. Boyle SC, Kim M, Valerius MT, McMahon AP, Kopan R. Notch pathway activation can replace the requirement for *Wnt4* and *Wnt9b* in mesenchymal-to-epithelial transition of nephron stem cells. *Development.* 2011; 138:4245–54. [PubMed: 21852398]
73. Ryan G, Steele-Perkins V, Morris JF, Rauscher FJ 3rd, Dressler GR. Repression of *Pax-2* by *WT1* during normal kidney development. *Development.* 1995; 121:867–75. [PubMed: 7720589]
74. Moore AW, McInnes L, Kreidberg J, Hastie ND, Schedl A. YAC complementation shows a requirement for *Wt1* in the development of epicardium, adrenal gland and throughout nephrogenesis. *Development.* 1999; 126:1845–57. [PubMed: 10101119]

75. Takemoto M, He L, Norlin J, Patrakka J, Xiao Z, Petrova T, et al. Large-scale identification of genes implicated in kidney glomerulus development and function. *EMBO J.* 2006; 25:1160–74. [PubMed: 16498405]
76. O'Brien LL, Grimaldi M, Kostun Z, Wingert RA, Selleck R, Davidson AJ. *Wt1a*, *Foxc1a*, and the Notch mediator *Rbpj* physically interact and regulate the formation of podocytes in zebrafish. *Dev Biol.* 2011; 358:318–30. [PubMed: 21871448]
77. Rothenpieler UW, Dressler GR. Pax-2 is required for mesenchyme-to-epithelium conversion during kidney development. *Development.* 1993; 119:711–20. [PubMed: 8187639]
78. Torres M, Gómez-Pardo E, Dressler GR, Gruss P. Pax-2 controls multiple steps of urogenital development. *Development.* 1995; 121:4057–65. [PubMed: 8575306]
79. Massa F, Garbay S, Bouvier R, Sugitani Y, Noda T, Gubler MC, et al. Hepatocyte nuclear factor 1beta controls nephron tubular development. *Development.* 2013; 140:886–96. [PubMed: 23362349]
80. Nakai S, Sugitani Y, Sato H, Ito S, Miura Y, Ogawa M, et al. Crucial roles of *Brn1* in distal tubule formation and function in mouse kidney. *Development.* 2003; 130:4751–59. [PubMed: 12925600]
81. Barker N, van Es JH, Jaks V, Kasper M, Snippert H, Toftgård R, et al. Very long-term self-renewal of small intestine, colon, and hair follicles from cycling *Lgr5*+ve stem cells. *Cold Spring Harb Symp Quant Biol.* 2008; 73:351–6. [PubMed: 19478326]
82. Barker N, Rookmaaker MB, Kujala P, Ng A, Leushacke M, Snippert H, et al. *Lgr5*(+ve) stem/progenitor cells contribute to nephron formation during kidney development. *Cell Rep.* 2012; 272:540–52. [PubMed: 22999937]
83. Brunskill EW1, Potter SS. RNA-Seq defines novel genes, RNA processing patterns and enhancer maps for the early stages of nephrogenesis: Hox supergenes. *Dev Biol.* 2012; 368:4–17. [PubMed: 22664176]
84. McMahon AP, Aronow BJ, Davidson DR, Davies JA, Gaido KW, Grimmond S, et al. Lessard. GUDMAP: the genitourinary developmental molecular anatomy project. *J Am Soc Nephrol.* 2008; 19:667–71. [PubMed: 18287559]
85. Mae S, Shono A, Shiota F, Yasuno T, Kajiwarra M, Gotoda-Nishimura N, et al. Monitoring and robust induction of nephrogenic intermediate mesoderm from human pluripotent stem cells. *Nat Commun.* 2013; 4:1367. [PubMed: 23340407]
86. Xia Y, Nivet E, Sancho-Martinez I, Gallegos T, Suzuki K, Okamura D, et al. Directed differentiation of human pluripotent cells to ureteric bud kidney progenitor-like cells. *Nat Cell Biol.* 2013; 15:1507–15. [PubMed: 24240476]
87. Taguchi A, Kaku Y, Ohmori T, Sharmin S, Ogawa M, Sasaki H, et al. Redefining the in vivo origin of metanephric nephron progenitors enables generation of complex kidney structures from pluripotent stem cells. *Cell Stem Cell.* 2014; 14:53–67. [PubMed: 24332837]
88. Takasato M, Er PX, Becroft M, Vanslambrouck JM, Stanley EG, Elefanty AG, et al. Directing human embryonic stem cell differentiation towards a renal lineage generates a self-organizing kidney. *Nat Cell Biol.* 2014; 16:118–26. [PubMed: 24335651]
89. Rinkevich Y, Montoro DT, Contreras-Trujillo H, Harari-Steinberg O, Newman AM, Tsai JM, et al. In vivo clonal analysis reveals lineage-restricted progenitor characteristics in mammalian kidney development, maintenance, and regeneration. *Cell Rep.* 2014; 7:1270–83. [PubMed: 24835991]
90. Appel D, Kershaw DB, Smeets B, Yuan G, Fuss A, Frye B, et al. Recruitment of podocytes from glomerular parietal epithelial cells. *J Am Soc Nephrol.* 2009; 20:333–43. [PubMed: 19092119]
91. Hackl MJ, Burford JL, Villanueva K, Lam L, Suszták K, Schermer B, et al. Tracking the fate of glomerular epithelial cells in vivo using serial multiphoton imaging in new mouse models with fluorescent lineage tags. *Nat Med.* 2013; 19:1661–6. [PubMed: 24270544]
92. Kusaba T, Lalli M, Kramann R, Kobayashi A, Humphreys BD. Differentiated kidney epithelial cells repair injured proximal tubule. *Proc Natl Acad Sci.* 2014; 111:1527–32. [PubMed: 24127583]
93. Berger K, Bangen JM, Hammerich L, Liedtke C, Floege J, Smeets B, et al. Origin of regenerating tubular cells after acute kidney injury. *Proc Natl Acad Sci.* 2014; 111:1533–1538. [PubMed: 24474779]

94. Ronconi E, Sagrinati C, Angelotti ML, Lazzeri E, Mazzinghi B, Ballerini L, et al. Regeneration of glomerular podocytes by human renal progenitors. *J Am Soc Nephrol*. 2009; 20:322–32. [PubMed: 19092120]
95. Angelotti ML, Ronconi E, Ballerini L, Peired A, Mazzinghi B, Sagrinati C, et al. Characterization of renal progenitors committed toward tubular lineage and their regenerative potential in renal tubular injury. *Stem Cells*. 2012; 30:1714–25. [PubMed: 22628275]
96. Villanueva S, Céspedes C, Vio CP. Ischemic acute renal failure induces the expression of a wide range of nephrogenic proteins. *Am J Physiol Regul Integr Comp Physiol*. 2006; 290:R861–R870. [PubMed: 16284088]
97. Cohen T, Loutochin O, Amin M, Capolicchio JP, Goodyer P, Jednak R. PAX2 is reactivated in urinary tract obstruction and partially protects collecting duct cells from programmed cell death. *Am J Physiol Renal Physiol*. 2007; 292:F1267–F1273. [PubMed: 17164400]

Highlights

- Nephrons are the filtering units of the kidney with distinct functional segments
- All segments of the nephron are derived from a common progenitor precursor
- Wnt signaling is critical to nephron induction
- Stromal cells contribute to nephron induction
- Notch signaling and other factors are essential to nephron patterning

Fig. 1. Regulation of nephron progenitor induction

The self-renewing nephron progenitors are demarcated by the expression of Six2 and Cited1. Six2 promotes self-renewal in addition to Wnt9b signals from the ureteric bud which directly promote expression of progenitor genes such as Cited1. nYap signaling may also cooperate with β -catenin induced from canonical Wnt9b signals to promote progenitor self-renewal. Bmp7-SMAD signaling promotes the conversion of nephron progenitors to a Six2+Cited1- state where they can be induced by Wnt9b and turn on differentiation markers Lef1 and Wnt4. These cells form the pretubular aggregate highlighted by the expression of critical differentiation factors Fgf8, Wnt4 and Lhx1. Foxd1+ stromal cells promote Bmp7-SMAD signaling in the nephron progenitors by repressing Dcn, an antagonist of Bmp7 activity. Stromal Fat4 regulates the induction process by inducing nuclear export and phosphorylation of Yap, which allows Wnt9b inductive signals to promote differentiation of the nephron progenitors. NP=nephron progenitors; UB=ureteric bud; SM=stromal mesenchyme; PTA=pretubular aggregate; dotted arrow represents promotion of self-renewal.

Fig. 2. Regulation of nephron patterning

Proximal/distal polarity is established in the renal vesicle and demarcated by the expression of several genes, including three Notch ligands: Dll1, Lfng and Jag1. The Notch pathway establishes a proximal polarity that is carried through the comma- and S-shaped body stages and integral for proximal tubule and podocyte development. Wt1 also promotes proximal fate, specifically that of the podocyte, by antagonizing Pax2, and cooperating with Notch pathway components and Foxc2, to regulate genes necessary for podocyte development. Endothelial cells are recruited by signals from developing podocytes of the S-shaped body. Hnf1b promotes proximal and intermediate/medial fate through regulation of Notch ligand expression and others factors such as Irx1/2 which may play a role in medial segment differentiation. Intermediate and distal fates are regulated by Brn1 which establishes distal polarity starting at the renal vesicle stage. Lgr5 is expressed in the distal segment of the comma-shaped body and the distal and intermediate segments of the S-shaped body, however a direct role in establishing or maintaining these segments has not been shown. Proximal polarity establishes the glomerulus and S1–S3 segments of the proximal tubule. Intermediate segments give rise to the loop of Henle. Distal segments establish the distal tubule, which hooks up to the collecting duct through a connecting segment. Inter=Intermediate; Prox=proximal; Podo=podocyte; ?=direct role not established, dashed arrow=ligand-receptor engagement.