Published in final edited form as:

Wiley Interdiscip Rev Dev Biol. 2013 September ; 2(5): 559–585. doi:10.1002/wdev.92.

Kidney organogenesis in the zebrafish: insights into vertebrate nephrogenesis and regeneration

Gary F. Gerlach¹ and Rebecca A. Wingert^{1,2}

¹Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556

Abstract

Vertebrates form a progressive series of up to three kidney organs during development—the pronephros, mesonephros, and metanephros. Each kidney derives from the intermediate mesoderm and is comprised of conserved excretory units called nephrons. The zebrafish is a powerful model for vertebrate developmental genetics, and recent studies have illustrated that zebrafish and mammals share numerous similarities in nephron composition and physiology. The zebrafish embryo forms an architecturally simple pronephros that has two nephrons, and these eventually become a scaffold onto which a mesonephros of several hundred nephrons is constructed during larval stages. In adult zebrafish, the mesonephros exhibits ongoing nephrogenesis, generating new nephrons from a local pool of renal progenitors during periods of growth or following kidney injury. The characteristics of the zebrafish pronephros and mesonephros make them genetically tractable kidney systems in which to study the functions of renal genes and address outstanding questions about the mechanisms of nephrogenesis. Here, we provide an overview of the formation and composition of these zebrafish kidney organs, and discuss how various zebrafish mutants, gene knockdowns, and transgenic models have created frameworks in which to further delineate nephrogenesis pathways.

Introduction

Vertebrate kidneys have multiple roles—they excrete waste, perform osmoregulation, reabsorb metabolites, balance acid-base levels, and even secrete hormones. Kidneys are comprised of many specialized cells in order to accomplish this panorama of diverse activities. In humans, for example, there are more than 20 differentiated renal cell types¹. Renal cells are broadly classified as parenchyma (functional cells) and stroma (support cells), and these categories include diverse epithelial and mesenchymal cell types. The epithelial cells are arranged into working units called nephrons, and the mesenchymal cells occupy the intervening interstitial spaces between nephrons. Nephrons consist of a blood filter, tubule, and duct, with each region showing a segmental organization in which subdomains of phenotypically distinct epithelia carry out specific physiological tasks¹. Nephrons filter blood plasma at their proximal end and convey the filtrate into the tubule. Within the tubule, a series of proximal, intermediate and distal tubule segments modify the passing filtrate to reabsorb and/or secrete solutes, ultimately draining the fluid waste into collecting ducts. Meanwhile, the interstitial cells produce hormones, and regulate dynamic flux of the local extracellular matrix. Vertebrate kidneys contain hundreds to millions of nephrons immersed in this complex tissue milieu, and thus are anatomically and functionally quite intricate organs.

²Corresponding Author: Rebecca A. Wingert, Ph.D., Assistant Professor, Department of Biological Sciences, Galvin Life Science Research Building, Rm. 218, University of Notre Dame, Notre Dame, IN 46556 USA, Tel: (574)-631-0907, rwingert@nd.edu.

During vertebrate ontogeny, kidney development is unique compared to that of other organs because multiple renal structures are generated and then degraded in the course of embryonic and/or larval life². Interestingly, each renal structure is constructed of similar nephron building blocks and derives from the intermediate mesoderm (IM)². Higher vertebrates, like mammals, form as many as three kidneys that sequentially arise from the IM: the pronephros, mesonephros, and metanephros. Each progressive renal structure is generally more elaborate than the previous one, and contains nephrons in differing numbers, arrangements, and even degrees of function. Kidney development has been extensively studied in several animal models, including fish, amphibians, birds and mammals². In particular, mammalian studies have provided significant advances in our understanding of IM specification and the processes of metanephros formation, such as how inductive signals between mesenchymal and epithelial components drive branching morphogenesis to create arborized duct and nephron arrays³. However, the complicated architecture of the mammalian metanephros has largely precluded detailed analysis of how renal precursors generate nephrons, and there is only a rudimentary knowledge about how the different nephron epithelial segments are patterned³.

The zebrafish has become an important model system for studying vertebrate development in recent years⁴. Methods including large-scale forward and reverse genetic screens, genomics, and chemical genetics have facilitated many discoveries about the pathways that regulate organogenesis⁴. The zebrafish kidney is now recognized as a valuable paradigm to identify and assess the function of renal genes and model renal diseases^{5–8}. During embryogenesis, the zebrafish forms a simple pronephros comprised of a pair of nephrons⁹, and will continue to use this kidney structure over several weeks of larval life (Figure 1). The zebrafish juvenile eventually develops a mesonephros, which contains several hundred nephrons and operates during ongoing larval growth and adulthood (Figure 1). Zebrafish, like other teleost fish, never manufacture a third, metanephric kidney. Intriguingly, the adult zebrafish kidney exhibits the property of neonephrogenesis, in which new nephrons are generated from clusters of renal progenitor cells as the fish grows in size or incurs renal damage, a trait not typically exhibited among higher vertebrates¹⁰. Several features of kidney development also differ between the zebrafish and other vertebrates. For example, the mammalian pronephros is comprised of vestigial nephrons, and the mammalian mesonephros functions transiently, and then gets degraded upon completion of the metanephros during gestation.

Regardless of these differences, the striking conservation of nephron building blocks across species makes the zebrafish pro- and mesonephros kidneys useful models to study the mechanisms of vertebrate nephrogenesis. Nephron progenitors in zebrafish express orthologous genes as other vertebrates, and their differentiated nephron epithelia share many cellular and molecular characteristics^{5–8}. In addition, the attributes of zebrafish development enable access to nephron development in ways that are not available in amphibian, avian or mammalian models. The external fertilization and optical transparency of the zebrafish embryo enable *in vivo* observation of pronephros development and function, which can be assessed by gene expression, transgenics and physiological assays. The early stages of zebrafish mesonephros formation can also be monitored *in vivo*, enabling the study of nephron growth and morphogenesis in more complex settings than the young embryo. Nephrogenesis during renal homeostasis and in response to injury can also be studied in both the zebrafish pro- and mesonephros, providing an opportunity to examine the biology of epithelial regeneration and renal stem cells. Taken together, the ability to study nephrogenesis processes in these whole-organism contexts provides a novel and powerful vehicle to delineate kidney development processes that complements other animal models.

This review presents the current understanding of zebrafish kidney development. Pronephros composition, patterning, differentiation and maturation are discussed. These sections are followed by a discussion of the processes involved in zebrafish mesonephros development, and maintenance of this kidney by neonephrogenesis and regeneration. Finally, some applications of knowledge about zebrafish kidney development for understanding and treating renal birth defects and kidney disease are presented.

PRONEPHROS SPECIFICATION AND PATTERNING

Embryonic origins and composition of nephrons in the pronephros—The zebrafish pronephros arises from bilateral stripes of a renal progenitor cell field that emerge from IM precursors. Fate mapping of shield stage embryos has indicated that pronephros cells derive from an area of the ventral mesoderm located near to and partly intermingled with blood and vascular progenitors^{11–12} (Figure 2, 3). Cell movements during epiboly and gastrulation distribute the IM into a prototypical ‘U’ shape by the tailbud stage, such that the IM is distributed in parallel tracks on either side of the midline that meet caudally in a semi-circular arc in the tailbud (Figure 2(a)). The IM is situated between the paraxial mesoderm (PM) that is fated to become the axial somites, and the lateral plate mesoderm (LPM) that occupies the farthest lateral position in the embryo. As development progresses through somitogenesis stages, the U-shaped IM territory becomes more elongated, and the parallel IM tracks become situated closer to the midline and each other^{13–16}. These morphological changes are likely due to a combination of cell movements and growth that lengthen the posterior body during axial extension of the trunk¹⁷, such as convergent extension movements that bring the nephrons into close apposition¹⁸.

Based on gene expression studies, the renal progenitor cell field that emerges from the IM at/during early somitogenesis stages is located adjacent to a field of cells that generates a mixture of angioblasts and primitive blood precursors^{19–23}. Angioblast precursors are situated in a lateral stripe next to the PM, with the blood precursors located in posterior stripes just lateral to the angioblasts, and the renal progenitor cell field located in the lateral-most position^{19–23} (Figure 2(b)). At the 5 somite stage, the vascular progenitors are thought to include cells that express the transcription factor *T-cell acute lymphocytic leukemia 1* (*tall*, formerly known as *stem cell leukemia (sch)*), while the hematopoietic progenitors are thought to encompass cells that co-express *tall* and the transcription factor *GATA binding protein 1a* (*gata1a*)^{19–23}. Markers of the renal progenitor cell field by the 5 somite stage include the *paired box* transcription factors *pax2a* and *pax8* as well as the gene *LIM homeobox 1a* (*lhx1a*, formerly known as *lim1*)^{13–16,24,25}. These genes continue to mark the renal progenitor field through the 15 somite stage^{13–16,24,25}.

As development proceeds past the 15 somite stage, the renal progenitor mesenchymal precursors embark on physical and morphological rearrangements that will create a pair of nephrons that are similarly situated in parallel tracks on either side of the trunk²⁵ (Figure 2(c)). The mesenchymal nephron precursors adopt an epithelial state (MET), undergoing tubulogenesis and forming a lumen²⁵. The process of nephrogenesis also involves the regional differentiation of the nephron precursors into spatially distinct segments of epithelial cell types²⁶. Segment boundaries are established by the 28 somite stage, based on analysis of dynamic gene expression profiles that fluctuate until this time^{26,27}. Nephron precursors in the rostral-most domain give rise to podocytes that form the glomerulus, and express the *Wilms tumor 1* (*Wt1*) paralogs *wt1a* and *wt1b*^{25–29}. Nephron precursors found in the remaining rostral, central, and caudal domains make a series of tubule segments that are followed by a duct^{26,27}. Rostral cells adjacent to the podocyte field develop into a neck segment, which connects the glomerulus and tubule, and is defined by expression of the ciliogenic transcription factor *regulatory factor X, 2* (*influences HLA class II expression*)

(*rfx2*)²⁶. The subsequent tubule consists of two proximal and two distal segments, which are defined by the non-overlapping expression domains of specific solute transporter genes^{26,27,30}. For example, the proximal convoluted tubule (PCT) segment expresses *solute carrier family 20, member 1a (slc20a1a)*, the proximal straight tubule (PST) expresses *transient receptor potential cation channel, subfamily M, member 7 (trpm7)*, the distal early (DE) expresses *solute carrier family 12, member 1 (slc12a1)*, and distal late (DL) expresses *solute carrier family 12 (sodium/chloride transporters), member 3 (slc12a3)*^{26,30}. The DL segments lead to bilateral pronephric duct (PD) segments, which are defined by expression of the transcription factor *GATA-binding protein 3 (gata3)*, and join with the cloaca (C) to make a common exitway²⁶. Also associated with the nephrons are the corpuscles of Stannius (CS), bilateral clusters of cells that arise in at the boundary between the DE and DL, and later become situated just dorsal to this region²⁶. The segmental organization of zebrafish pronephric nephrons follows the archetypal pattern of proximal and distal domains that is broadly conserved across vertebrates ranging from amphibians to humans, though they lack an equivalent intermediate or thin limb segment found in mammals that facilitates urine concentration⁵.

Morphogenesis events that transpire between 24 and 48 hpf will create a functioning pronephros. These events include glomerulus formation, which involves the migration of podocyte precursors to the midline where they recruit vasculature and form a composite blood filter that is shared by the pair of nephrons. In addition, the PCT becomes coiled and the tubule segments experience regional growth over the next few days. Although the embryonic zebrafish pronephros is architecturally rather simple, with just this pair of nephrons, it is sufficient to support growth for several weeks of larval life, at which point a mesonephros kidney will begin to form. In the following sections, we discuss what is known about the signals that direct formation of the IM field, the processes of making each nephron cell type, and the control of nephron morphogenesis events.

Specification and early patterning of the pronephros renal progenitor cell field

—The events that direct mesoderm development are essential for proper IM formation and subsequent delineation of the renal progenitor cell field. Mesoderm development is closely intertwined with the patterning events that establish the embryonic axes, and alterations in mesoderm formation have dramatic consequences on whether or not the renal progenitor field forms, and where the field is situated along the anterior-posterior axis (Figure 3).

Mesoderm induction and D-V patterning: Ventral mesoderm development is dependent on dorsoventral (D-V) pattern formation and the proper establishment of the embryonic axes (Figure 3(a)). The vertebrate D-V axis is established through events that lead to regionalized activity of members of the bone morphogenetic proteins (BMP) family that signal through their receptors to activate members of the SMAD family of transcription factors. High levels of BMPs specify ventral fates and lead to mesoderm induction. BMP activity is antagonized by inhibitory factors in dorsal regions that are secreted by cells of the organizer and enable the formation of dorsal tissues. Disruptions of normal ventral development, in which embryos display a dorsalized phenotype, lead to the reduction or abrogation of the mesoderm that includes the renal progenitor cell field, as gauged by *pax2a* expression³¹. Zebrafish embryos with mutations in the BMP ligands encoded by *bmp2b* (formerly known as *swirl (swr)*)^{32,33} and *bmp7a* (formerly known as *bmp7/snailhouse (snh)*)³⁴ show the most severe reductions in the *pax2a*-expressing cells, while those with mutations in the BMP receptor *activin A receptor, type 1 like (acvr1l)*, formerly known as *alk8/lost-a-fin/mini fin*^{35–37} and the signaling target *smad5* (formerly known as *somitabun (sbn)*/*piggytail (pgy)*)³⁸ show more mild reductions³¹. Alterations in dorsal development also effects changes in pronephros ventral fates, as evidenced by gain- and loss-of-function studies with the extracellular BMP antagonist Chordin^{39–41}. The overexpression of *chordin*

promotes dorsal fates at the expense of *pax2a*-expressing cells³⁹, while a truncation in Chordin encoded by the *dino*⁴⁰ mutation is associated with an enlarged domain of *pax2a*-expressing cells⁴¹.

T-box factors and mesoderm specification: Further elaboration of pronephros fates requires the activity of T-box transcription factors, which serve essential roles in directing the formation of the trunk and tail mesoderm in vertebrates (Figure 3(b)). *no tail a (ntla)* is the zebrafish ortholog of the mammalian Brachyury (or T) gene, a T-box transcription factor required for mesoderm development⁴². *ntla* is expressed in response to mesoderm induction by cells in the notochord and tailbud, and directs a mesoderm gene regulatory network that affects morphogenetic movements, posterior identity, muscle and notochord specification, and left/right patterning⁴³. *ntla* target genes are numerous and include other T-box factors like the gene *tbx16* (formerly known as *spadetail (spt)*)⁴³, which in turn regulates the induction of trunk mesoderm cell fates and convergence movements^{44,45}. While the loss of *ntla* or *tbx16* alone does not abrogate the renal progenitor cell field, *ntla/tbx16* doubly-deficient embryos fail to form renal progenitors along with other mesodermal derivatives, suggesting that these genes act synergistically and/or in a redundant fashion to promote pronephros fates⁴⁶.

cdx genes and axial positioning of the pronephros field: The proper positioning of the zebrafish pronephros along the embryonic anterior-posterior (A-P) axis requires the partially overlapping activities of the *caudal type homeobox (cdx)* transcription factors (Figure 3(c)). In zebrafish, *cdx4* and *cdx1a* function redundantly across the germ layers to confer segmental identities along the A-P axis by influencing the expression domains of *homeobox (hox)* transcription factors, and also control posterior body elongation^{21,47–49}, roles conserved with the activities of *Cdx1*, *Cdx2*, and *Cdx4* in mammals⁵⁰. The progressive loss of *cdx* function leads to posterior body truncations and shifts the A-P position of the renal progenitor cell domain^{21,26,48}. For example, the rostral boundary of the renal progenitor cell field normally develops adjacent to the third somite, but *cdx4*-null embryos exhibit a posteriorly shifted domain that is situated adjacent to the fifth somite, and *cdx4/1a* double-deficient embryos have a further posterior shift of the domain back to the seventh somite²⁶. Interestingly, the loss of *cdx1a* alone is not sufficient to alter axial positioning of the renal progenitor cell field^{26,48}. The posterior shift in the nephron progenitor field in *cdx4*-null embryos is also associated with the truncation of distal segments, though each nephron segment is formed²⁶. In contrast, the severe posterior body truncation in *cdx4/1a*-deficient embryos is associated with a loss of multiple segments that include the PST and all distal segments, and the tubules fail to fuse and form a cloaca²⁶. Nephron segment loss in *cdx4/1a*-deficient embryos is likely influenced by their axial elongation phenotype, and thus may not reflect a direct requirement for *cdx* genes in patterning distal nephron segments²⁶. It is unknown whether *cdx* genes mediate positioning of the renal progenitor cell field through the global regulation of *hox* genes in the mesoderm or other germ layers, though this notion has merit based on the precedence of *cdx* activity in other developing tissues²⁶.

Maintenance of the pronephros field: The activity of the zinc finger transcription factor *odd-skipped related 1 (Drosophila) (osr1)* gene is essential to maintain proximal nephron fates (Figure 3(c)). *osr1* is expressed in the mesendoderm during gastrulation, then later during somitogenesis is expressed in bilateral stripes of lateral plate cells adjacent to the renal progenitor cell field^{51,52}. Knockdown of *osr1* leads to a specific loss of podocytes and deleted stretches of the PCT^{51,52}. The renal progenitor cell field is unperturbed in *osr1* morphants through the 5 somite stage based on *pax2a* expression, suggesting that pronephros specification proceeds normally⁵². However, *osr1* morphants show reductions in the rostral IM expression domains of *pax2a* and *lhx1a* beginning at the 14 somite stage, and

display a concomitant expansion in the *tall1*+ angioblast population^{51,52}. *osr1* knockdown also causes an expansion of the endoderm, and surprisingly *pax2a* expression can be rescued in *osr1* morphants when the endoderm is reduced by abrogating activity of the essential endoderm transcription factors *SRY-box containing gene 32* (*sox32*, formerly known as *casanova* (*cas*)) or *mixer* (formerly known as *bonnie and clyde* (*bon*))⁵². These findings highlight a role for endoderm-derived signal(s) in pronephros maintenance. These data also suggest that *osr1* could directly maintain the pronephros field by restricting angioblast expansion or blocking the conversion of pronephros cells to alternative fates⁵². As *pax2a* overexpression is sufficient to rescue proximal fates in *osr1* morphants, *pax2a* levels appear to be the lynchpin for pronephros maintenance in the absence of *osr1* activity⁵².

Interestingly, the inhibition of WNT signaling during early somitogenesis leads to deletions of the proximal nephron that are reminiscent to the phenotype of proximal tubule abrogation in the setting of *osr1*-deficiency⁵³. Using a heat-shock inducible Dkk1 construct, WNT/ctnnb2 (formerly known as β -catenin) signaling was inhibited during embryogenesis by a series of heat shocks initiated at the 12 somite stage, which led to variably-sized deletions of the proximal tubule in 72 hpf embryos⁵³. Further analysis is needed to pinpoint when and how the proximal domain is eliminated by WNT/ctnnb2 deficiency. One intriguing possibility is that WNT/ctnnb2 signaling acts similarly to *osr1* and maintains the pronephros field by regulating targets like *pax2a*.

Patterning of the proximo-distal nephron segment identities—Nephron segmentation is the process through which regional identities emerge during nephron formation. Numerous gene expression patterns within the renal progenitor field fluctuate during somitogenesis stages in the time period before the emergence of nephron segments. Some key events and molecular signals that dictate nephron segmentation have been identified, but many questions remain (Figure 4, 5). For example, the action(s) of retinoic acid (RA) mediate the number of renal progenitor cells and their subdivision into broad rostral and caudal domains, though how these domains are regionalized is poorly understood. Recent work has provided some insights into the signals that direct the differentiation of the podocyte lineage. The events that act to pattern the respective tubule segments are largely unknown, with only a handful of essential components identified to date.

RA patterning of the pronephros field: During early somitogenesis, the renal progenitor cell field exhibits subdivisions into multiple domains of nephron progenitors based on gene expression and fate-mapping studies^{26–28} (Figure 4(a–c)). At the 5–6 somite stage, a broad rostral domain is located adjacent to somites 2–5, defined by the expression of the Notch ligand genes *deltaC* (*dlc*) and *jagged 2* (*jag2*)^{26,27}, though notably a subset of cells located adjacent to somites 2–3 also express *wt1a* and are fated to the podocyte lineage^{26,28}. A broad caudal domain begins adjacent to somite 6 and is marked by expression of the zinc finger transcription factor gene *MDS1 and EVI1 complex locus* (*mecom*, formerly known as *evi1*)^{26,27}. While these rostral/caudal domains initially appear to be mutually exclusive (Figure 4(b)), by the 8 somite stage the *jag2* domain expands caudally, creating a nested pattern of overlapping rostral and caudal territories among the renal progenitor cell field²⁷ (Figure 4(c)). The significance of these early subdivisions has yet to be elucidated. However, changes in the rostral and caudal territories strongly correlate with alterations in the production of the proximal and distal segments, respectively^{26,27}.

Several lines of evidence suggest that patterning of the renal progenitor cell field into rostral/caudal domains is reliant on RA signaling, which enables the development of proximal identities from the rostral domain^{26,27}. Embryos deficient for RA biosynthesis, either because of genetic defects in *aldehyde dehydrogenase 1 family, member A2* (*aldh1a2*,

formerly known as *raldh2/neckless (nls)/no-fin (nof)* or chemical treatment with diethylaminobenzaldehyde (DEAB) that blocks Aldh enzymatic activity, show a loss of the rostral domain and later fail to form podocytes and proximal tubule segments^{26,27}. The abrogation of proximal fates is associated with a concomitant expansion of the caudal domain, then later expanded distal tubule and duct segments^{26,27}. Conversely, exposure to exogenous RA leads to an expanded rostral domain and proximal segments, while both the caudal domain and distal segments are reduced²⁶. These results indicate that RA is essential to induce proximal fates and may inhibit distal fates (Figure 5). Of note, the strongest evidence is that RA induces podocytes along with transporting epithelia in the PCT and PST, while inhibiting formation of CS cells and transporting epithelia in the DE, DL and PD; additional marker analysis is needed to better evaluate the effects of RA on other cell types like those found in the neck segment. The most dramatic alterations in proximo-distal segment patterning result from modulation of RA levels between the end of gastrulation and early somitogenesis stages, and are associated with alterations in the rostral/caudal IM domains that are detected as early as the 8 somite stage^{26,27}. For example, DEAB treatment abolishes the rostral IM domain, while mutants with diminished *aldh1a2* activity have a shortened rostral IM domain and expanded caudal IM^{26,27}. These findings suggest that RA exerts early, dosage-sensitive effect(s) that regionalize the renal progenitor cell field. Further, these data imply that the renal progenitor cell field is responsive to alterations in RA signaling over this time^{26,27}.

RA is a well-established morphogen that exerts concentration-dependent patterning effects that are controlled by its production and degradation in developing tissues⁵⁴. During the time-window when the renal progenitor cell field is responsive to RA, the anterior (or cervical) PM secretes RA (Figure 4(a)). Evidence that the PM provides a significant source of the RA that patterns the renal progenitor cell field was provided by knockdown studies of *tbx16*²⁷. Abrogation of *tbx16* reduces PM development and dramatically reduces *aldh1a2* expression in the PM field at the 5 somite stage; later, these changes are associated with shortened proximal and expanded distal segments²⁷. Interestingly, the mechanism by which *cdx* deficiency alters renal progenitor cell field positioning appears to be related to the axial location of PM-produced RA activity in the trunk tissues during early somitogenesis. Progressive *cdx*-deficiency correlates with posterior shifts in the domains of *aldh1a2* expression and the RA-degradation enzyme encoded by *cytochrome P450, subfamily XXVIA, polypeptide 1 (cyp26a1)*, suggesting that a swath of 'bioavailable' RA is shifted posteriorly along the trunk when Cdx function is absent²⁶. Additional studies are needed to identify non-PM sources of RA, and to gain a better understanding of how the dynamics of these so-called RA 'sources and sinks' influence nephron patterning. Notably, distal nephron cells express *aldh1a2* transcripts at later somitogenesis stages^{55,56}, and exhibit activity in an RA transgenic reporter at 24 hpf⁵⁷. These data suggest that RA might play additional role(s) in nephrogenesis at later timepoints, and that RA secretion by the pronephros may even be one such mechanism.

Further insights into another dimension of how RA influences nephron progenitor development have been provided by studies that revealed the effects of histone deacetylase (HDAC) inhibitors on the renal progenitor cell field. Treatment with the HDAC inhibitors 4-phenylthiobutanoic acid (PTBA), 4-phenylbutanoic acid (PBA), or trichostatin A (TSA) starting at 2 hpf leads to a concentration-dependent expansion of the renal progenitor cell field by the 10 somite stage⁵⁸. The expansion of renal progenitors was diminished in the embryo when a dominant-negative *retinoic acid receptor* (DN-RARα) was overexpressed⁵⁸. This finding suggests that HDAC activity influences renal progenitor cell field numbers by mediating RA signaling⁵⁸.

Dynamicism of gene expression among tubule segment progenitors: The mechanisms that direct formation of each specific tubule and duct segment in the zebrafish pronephros remain largely enigmatic. Many transcription factors and signaling pathway components are expressed in the renal progenitor cell field subsequent to the appearance of the rostral/caudal subdomains at the 5–8 somite stage²⁷. By the 15 somite stage, the rostral/caudal subdomains are further elaborated into a complex series of overlapping subdomains of nephron progenitors²⁷ (examples shown in Figure 4(d)). The gene expression domains appear to follow a nested array of multiple rostral, central, and caudal domains, marked by such transcription factor genes as *hepatocyte nuclear factor 4, alpha (hnf4a)*, the *iroquois homeobox protein 3b (irx3b)*, and *gata3*, respectively²⁷. The nested pattern is both intricate and highly dynamic during nephrogenesis. The nephron progenitors exhibit protracted fluctuations in gene expression between the 15 and 28 somite stages, and many transcription factor domains are progressively restricted to smaller domains (Figure 4(e))²⁷. The gene expression changes do not appear to reflect substantive alterations in renal cell proliferation, though it is conceivable that cell turnover may contribute prior to or at the 15 somite stage²⁷. Future studies are needed to piece together if and how the changing combinations of gene expression influence nephron segmentation and/or epithelial differentiation. To date, the transcription factors *pax2a* and *irx3b* have been demonstrated to play important roles in nephrogenesis (discussed below).

pax2a-mediated negotiation of the podocyte and neck boundaries: There is evidence that *pax2a* mediates whether the neck segment emerges from the rostral-most renal progenitor cell field. In the *pax2a* mutant *no isthmus (noi)*, the absence of *pax2a* function leads to ectopic expression of *wt1a* transcripts at 24 hpf in the region where the neck segment should form⁵⁹. At later timepoints, these cells fail to express the Na⁺/K⁺ ATPase protein that normally marks tubule epithelium, and instead express *vascular endothelial growth factor Aa (vegfaa)*, formerly known as *vegfa*, a marker of differentiating podocytes⁵⁹. These phenotypes suggest that podocyte gene programs have been implemented in lieu of neck tubular segment programs (Figure 5). One explanation for this outcome is that *pax2a* acts directly to inhibit podocyte formation, possibly by antagonizing *wt1a* activity.

Recent insights into the regulation of *pax2a* were shown in a study that found the gene *plac8 onzin related protein 1 (ponzr1)* is an essential *pax2a* modulator⁶⁰ (Figure 5). Knockdown of *ponzr1* disrupts nephron patterning such that *pax2a* is ectopically expressed across the midline at the 18 somite stage, rather than being spatially restricted to regions on either side of the midline⁶⁰. The ectopic cells at the midline activate the expression of the podocyte markers *wt1a* and *nephrosis 2, idiopathic, steroid-resistant (podocin) (nphs2)*, formerly known as *podocin*, however they continue to express *pax2a* transcripts through 72 hpf and do not differentiate properly to form a functioning glomerulus⁶⁰. These findings indicate that *pax2a* downregulation is essential for podocyte development⁶⁰. Taken together, it is evident that *pax2a* spatial localization and expression level are crucial to resolve the fates of both podocytes and neck cells.

DE segment formation and irx3b: A number of the regionalized gene expression domains that typify the zebrafish nephron progenitors between the 15–28 somite stages have been reported during nephrogenesis in other vertebrates⁵. Among these, developing nephrons in the amphibian pronephros and mammalian metanephros exhibit a centralized expression domain of the *irx3b* orthologue *Irx3*⁶¹. Further, knockdown studies of *Irx3* in the *Xenopus* pronephros⁶¹ and *irx3b* in the zebrafish pronephros²⁷ have identified a conserved requirement for *Irx3/3b* function to form *slc12a1+* distal tubule cells (which correspond to the DE segment in zebrafish) (Figure 5). Interestingly, zebrafish nephron progenitors at the 15 somite stage express *irx3b* transcripts in a domain that likely includes cells that contribute to a caudal portion of the PCT, the PST, and the DE²⁷. Abrogation of *irx3b*

activity using morpholinos eliminates the DE segment, based on the loss of DE segment-specific solute transporter gene expression, and *irx3b* morphants form expanded PCT and PST segments²⁷. These findings suggest that *irx3b* activates a DE gene program, and may play roles in negotiating the PCT/PST boundary²⁷. Continued loss- and gain-of-function analysis for other transcription factors expressed in the renal progenitors is needed to ascertain their roles and also their relationships to *pax2a*, *ponzr1*, and *irx3b*.

PRONEPHROS MATURATION: CELL DIFFERENTIATION AND MORPHOGENESIS

Making the glomerulus—The mature vertebrate glomerulus is a blood filter apparatus made up of vascular endothelium and renal cells called podocytes⁶². At the center of the glomerulus is a capillary of fenestrated endothelial cells, which are surrounded by the podocyte epithelial cells. Between these cell layers is a glomerular basement membrane (GBM), which is a composite of basement membranes from the endothelial and podocyte cells and serves as part of the sieve for the incoming filtrate. Mature podocytes extrude long cellular extensions from their basal surface along the GBM that become arborized at their terminal cell ends, known as foot processes. Neighboring podocytes interdigitate their foot processes and form unique junctional complexes, the slit diaphragm (SD), which further restricts passage of large macromolecules and cells into the nephron.

Although it has been long appreciated that the podocytes derive from the renal progenitors that make the nephron, the pathways that direct podocyte specification and differentiation are not well understood⁶². Studies of podocyte formation in zebrafish have provided some new insights into the molecular pathways of podocyte progenitor development and differentiation, many or all of which may be conserved with higher vertebrates. Podocyte development in the zebrafish pronephros is known to involve the coordination of instructions from RA, transcription factor activity, and Notch signaling that elaborate the podocyte identity from the renal progenitor cell field (Figure 5).

Further delineation of the podocyte lineage and interrenal gland precursors: Zebrafish podocyte progenitors are specified early after IM formation²⁸ and arise as bilateral populations adjacent to somite 3 that initially co-express *pax2a* along with *wt1a* and *wt1b*^{28,29} (Figure 6). While *wt1a* transcripts are found in broad bilateral cell domains²⁸, *wt1b* is expressed slightly later in development, with transcripts detected in bilateral cell clusters within the renal progenitor cell field that appear at the 15 somite stage and persist throughout podocyte migration and glomerulus formation²⁹ (Figure 4(b–e)). The overlapping *wt1a*+ and *wt1b*+ cells are believed to be podocyte progenitors²⁹. Podocyte progenitors at the 15 somite stage are also marked by the expression of the transcription factors *forkhead box C1a* (*foxc1a*), *lhx1a*, *v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B* (*mafba*, formerly known as *maf*), and the transcriptional repressor *hairly/enhancer-of-split related with YRPW motif 1* (*hey1*)^{63,64}. As somitogenesis progresses, podocytes sequentially express additional mature marker genes that will enable them to form intercellular junctions of the SD, such as *nephrosis 1, congenital, Finnish type (nephrin)-like* (*nphs1*, formerly known as nephrin) and podocalyxin-like (*podxl*) at 24 hpf, followed by *nphs2* and integrin, alpha 3a (*itga3a*) at 36 hpf^{64,65}.

The identity and fates of the cells that express only *wt1a* is yet to be fully resolved. However, by 20–22 hpf a scattered population of unique cells emerges in the same region as the bilateral *wt1a*+ fields, and these cells express a Ftz-F1 member of the nuclear receptor superfamily known as *nuclear receptor subfamily 5, group A, member 1a* (*nr5a1a*, formerly termed *SF1* and *ff1b*)⁶⁶ (Figure 6(b)). The *nr5a1a*+ cells give rise to the interrenal gland, a midline cluster of cells that will be situated posterior to the podocytes in embryonic stages⁶⁶. Between 22–30 hpf, the scattered *nr5a1a*+ cells undergo morphogenetic movements,

assembling into one centralized cluster at the midline^{66,67} (Figure 6(c–d)). Once formed, the interrenal gland functions as an endocrine organ that is held to be the teleostean counterpart of the mammalian adrenal gland^{66,67}. Interrenal and adrenal glands share the trait of being a major site for steroid synthesis^{66,67}. The zebrafish embryo interrenal organ is nestled just caudal to the glomerulus and shows expression of steroidogenic genes concomitant with formation of the centralized interrenal cluster^{66,67} (Figure 6(e)).

The differential expression pattern of the *wt1* paralogs suggests the idea of their potential sub-functionality, which has been tested in morpholino studies. Knockdown of *wt1a* leads to a reduction in podocyte number, and podocytes fail to express mature markers^{63,68}. These data provide one explanation for the mechanism of podocyte loss in RA deficiency, as reductions in RA lead to the abrogation of *wt1a* expression^{26,27}. Analysis of the *wt1a* enhancer in zebrafish and the human *Wt1* enhancer has also uncovered the presence of conserved motifs that elicited RA-responsiveness. The zebrafish *wt1a* and human *Wt1* enhancers were shown to bind heterodimer complexes of the retinoic acid receptor (RAR) transcription factors and their partner protein retinoid X receptor (RXR) *in vitro*, providing strong evidence that RA can directly regulate *wt1a/Wt1* transcription⁶⁹. The role of *wt1b* remains controversial. One study has reported that *wt1b* knockdown causes a subtle reduction in the expression of mature podocyte gene transcripts, which could indicate reduced podocyte number or a block in their differentiation⁶⁸. *wt1b* morphants were also observed to develop fluid-filled cysts in the glomerular region and pericardial edema, suggesting that their renal function is compromised over time⁶⁸. However, co-injection of *wt1a* and *wt1b* morpholinos was also reported to show a phenotype resembling *wt1a* knockdown, suggesting the podocytes in *wt1a*-deficiency arise independent of *wt1b* function⁶⁴. Taken together, these findings support the notion that *wt1a* and *wt1b* may play discrete roles in podocyte formation and maintenance.

Mechanistic insights into how *wt1a* establishes podocyte fates in the pronephros were recently suggested by studies of *wt1a*, *foxc1a* and the Notch transcriptional mediator *recombination signal binding protein for immunoglobulin kappa J region* (*rbpj*). While previous research has shown that activity of the forkhead transcription factor FoxC2 and the Notch pathway are required for vertebrate podocyte development, the coordination of their activities has remained unknown^{70–76}. Gene expression domains during early zebrafish somitogenesis are consistent with activity of a related forkhead factor, *foxc1a*, and Notch signaling in rostral IM progenitors. Transcripts encoding the *dIC*, *jagged 1b* (*jag1b*, formerly known as *jag3*) and *jag2* ligands are found in rostral IM domains that overlap or partially overlap with the *wt1a/b+* podocyte field^{26,27,77,78}, *notch1a* and *notch3* receptor transcripts are located throughout the IM⁷⁹, and *rbpja* and *rbpjb* transcripts show a ubiquitous distribution^{80,81}. Morpholino knockdown of *wt1a*, *foxc1a*, or *rbpja/b* reduces *wt1b+* podocytes at 15 somites⁶⁴. A genetic interaction between several of these factors was unveiled by the finding that dual knockdowns were needed to entirely eliminate podocytes⁶⁴. In biochemical assays including GST-pull-downs and co-immunoprecipitation experiments, zebrafish Wt1a, Foxc1a, the intracellular domain of Notch 3 (NICD3), and Rbpj were found to exhibit physical interactions⁶⁴. Based on this, these proteins are posited to coordinate podocyte development by forming a multimeric transcriptional complex⁶⁴.

Further, in light of the temporal sequence of podocyte gene expression changes during development, it has been hypothesized that the Wt1a/Foxc1a complex acts in a temporally and stoichiometrically dependent fashion during zebrafish podocyte development, such that its molecular composition undergoes changes congruent with the developing maturity of podocytes⁶⁴. In podocyte progenitors, the complex is proposed to rely on high levels of Notch signaling to regulate Notch responsive transcriptional targets such as *hey1*⁶⁴. As podocytes mature, an elevation in *wt1a* levels is proposed to alter the composition of the

complex and enable transcriptional activation of targets like the mature podocyte components such as the SD protein *nphs1*⁶⁴. Whether this model applies to mammalian podocyte development is unclear. Interestingly however, different combinations of murine Rbpj, Wt1, Foxc12, and NICD of Notch 1 (NICD1) were found to elicit different levels of promoter activation on murine Hey1, Podocalyxin and a synthetic Notch reporter *in vitro*⁶⁴. These data support the speculation that physical complexes of these proteins work to coordinate mammalian podocyte development, which certainly provides an intriguing model regarding the molecular circuitry of podocyte development that warrants further study.

The number of interrenal gland progenitors is also influenced by alterations in *wt1a* and *rbpj* expression levels⁶⁴. Both *wt1a* and *rbpj* morphants form an increased number of *nr5a1a*+ interrenal progenitors, and *wt1a/rbpj* double-deficiency caused an even greater expansion⁶⁴. In contrast, changes in *foxc1a* expression alone did not have effects on *nr5a1a*+ interrenal progenitors, but *wt1a/foxc1a* double-deficiency led to the abrogation of *nr5a1a*+ interrenal progenitors⁶⁴. These findings illustrate that a complex interplay between Wt1a, Foxc1a and Rbpj may also act to establish podocyte versus interrenal cell identity. Whether these factors collaborate to control an interrenal transcriptional network has yet to be explored, but it is reasonable to hypothesize that they work in different combinations and/or with additional partners to mitigate the interrenal fate decision.

Glomerulus assembly and terminal podocyte differentiation events: Glomerular development is reliant on migration of podocyte precursors to a midline position, where they coalesce to form a single glomerulus between 30–40 hpf²⁵ (Figure 6). Heart defects that lead to an absence of blood circulation also disrupt glomerulogenesis, and are associated with the failure of proper podocyte migration⁸². The podocytes complete differentiation at the midline. As the podocytes differentiate, vascularization commences in response to the production of VEGF by podocytes, which recruits endothelial cells from the nearby dorsal aorta⁸². Podocyte migration is regulated by midline signaling that originates from the notochord, as shown by analysis of the zebrafish mutants *floating head* (*flh*), *sonic hedgehog a* (*shha*, formerly known as *sonic you* (*syu*)) and *GLI-Kruppel family member GLI2a* (*gli2a*, formerly known as *you-too* (*yot*))⁸³. Podocyte migration fails to occur in *flh* mutants, which lack a notochord and dorsal aorta due to deficiency of the *flh* notochord specific helix loop helix transcription factor. While notochord formation occurs in *shha* and *gli2a* mutant embryos, these mutations both reduce Shh signaling and the transcriptional consequence in responding tissues. In these midline mutants, podocytes remain at lateral positions⁸³. Interestingly, *flh* podocytes express VEGF, and become surrounded by endothelial cells, suggestive that *flh* podocytes can recruit endothelium from some non-aortic origin⁸³. *flh* mutants also show evidence of glomerular filtration, based on fluorescent dextran uptake⁸³.

While the recruited endothelial cells secrete extracellular matrix that contributes to GBM production, there is evidence that suggests podocyte differentiation is somewhat uncoupled from the events of vascularization. In the *cloche* (*clo*) mutant, which lacks endothelial cells, podocytes attach to the basement membrane via extended foot processes that interdigitate with neighboring podocytes at 48 hpf⁸⁴. These data suggest that aspects of podocyte differentiation proceed autonomously. However at 48 and 96 hpf, sites of podocyte effacement were detected, suggesting that GBM integrity and/or podocyte maintenance were compromised at later time points in *clo* embryos⁸⁴. Interestingly, podocytes and endothelial cells share expression of the gene *myosin, heavy polypeptide 9a, non-muscle* (*myh9a*), which encodes the non-muscle myosin IIa (NMHC-IIA) protein⁸⁵. Knockdown of zebrafish NMHC-IIA led to multiple glomerular defects that included a failure of endothelial cells to develop fenestrations, a thickened GBM, irregular foot processes, and was associated with reduced glomerular filtration. These findings suggest that multiple glomerular cell types may utilize common genes for their normal development.

Differentiated podocytes have a number of discrete features, and podocyte differentiation involves the establishment of epithelial apical-basal polarity, attachment of the podocyte and foot processes to the GBM, and the proper expression and formation of the SD to construct a high-fidelity sieve. Some aspects of the epithelial character of zebrafish podocytes are reliant on cell polarity traits that are mediated by *crumbs homolog 2b (crb2b)*⁸⁶. *crb2b*, a conserved member of the apical-basal polarity complex, is expressed by podocytes beginning at 48 hpf and after the onset of glomerular filtration⁸⁶. Morpholino knockdown of *crb2b* was associated with irregular foot process extension, the misexpression and dislocalization of Nephtrn, and podocyte effacement⁸⁶. Podocytes in *crb2b* morphants show a normal relative orientation of the cell body/nucleus and have a normal GBM. These data suggest that *crb2b* is not required to establish overall apical-basal polarity, but rather refines the podocyte polarity to promote proper nephrin localization and facilitate foot process maturation. Further insight into *crb2b* is suggested by the finding that Crb proteins can bind the FERM domain protein Erythrocyte membrane protein band 4.1 like 5 (Epb4115, which is disrupted in the zebrafish mutant *mosaic eyes (moe)*)⁸⁷. Podocytes in *epb4115*-deficient mutants were found to form abnormal cell extensions from their apical surface⁶⁵. These observations support the hypothesis that *epb4115* and *crb2b* may cooperate in refining podocyte epithelial character⁸⁶.

Podocytes must make and maintain attachments to the GBM and neighboring cells to regulate glomerular integrity. To perform these tasks, podocytes are reliant on a branched actin cytoskeleton, which is thought to provide mechanical support for the extension of their membrane and also for adherence to the GBM. This specialized architecture is fundamentally important for podocyte function. Disruption of an actin associating protein, Cofilin-1, demonstrates a reduced SD integrity and podocyte effacement and fusion⁸⁸. Furthermore, interference in production of the SD components compromises glomerular integrity and disrupts normal foot process formation, as shown by knockdown of *nphs11* and *nphs2*⁶⁵. Interestingly, glomerular integrity was recently discovered to depend on expression of the amino acid transporter *solute carrier family 43, member 1a (slc43a1a)*, formerly known as the *system L amino acid transporter 3, lat3*⁸⁹. Slc43a1a localizes to the apical plasma membrane of podocyte foot processes⁸⁹. Knockdown of *slc43a1a* during zebrafish development led to glomerular collapse and a thickened GBM, but precisely how this transporter contributes to podocyte physiology remains unclear⁸⁹. Knockdown of a growing number of diverse proteins disrupts glomerular function and/or is associated with podocyte foot process effacement^{90,91}, suggesting that glomerular integrity involves a broad spectrum of cellular pathways that have only begun to be appreciated.

Tubule epithelial differentiation: the establishment of apical-basal polarity and regional functionality—The pronephros nephron tubules are comprised of four abutting segments of transporting epithelia followed by the pronephric duct. The boundaries of these mutually exclusive segments are established by the 28-somite stage, by which time the IM cells have also transitioned from a loose mesenchyme into an epithelial tube with a lumen at its center and ECM attachment at the basal surface²⁵ (Figure 7(a–b)). However, gene expression data suggest that the differentiation of the epithelial populations continues to occur over the second day of development²⁶. Each segment exhibits the expression of additional solute transporter genes, which likely reflects epithelial maturation/terminal differentiation events²⁶. By 48 hpf, the differentiated tubule epithelia exhibit additional polarized features, forming a primary cilium or multiple cilia, and fluid flow is established upon the onset of glomerular filtration (Figure 7(c)).

Epithelial polarity: shared and unique features among tubule segments: During differentiation, nephron epithelial cells acquire a distinct apical-basal polarity and show the fundamental features associated with this polarized state that are common across vertebrates.

The characteristics of a polarized epithelium include the apical localization of junctional complexes, actin, and adaptor complexes such as the ternary polarity complex, generated by the proteins encoded by *protein kinase C, iota* (*prkci*, formerly known as *aPKC*), *par-3 partitioning defective 3 homolog* (*C. elegans*) (*pard3*, formerly known as *par3*), and *par-6 partitioning defective 6 homolog alpha* (*C. elegans*) (*pard6a*). The pronephros tubules have been shown to exhibit apical surface localization of Prkci⁹². One protein that shows a basolateral membrane restriction is Na⁺/K⁺ ATPase²⁵. Disruption of apical-basal protein localization has been observed in a number of mutants and gene knockdowns. For example, a disruption in Na⁺/K⁺ ATPase basal localization was seen after morpholino knockdown of the *Rho guanine nucleotide exchange factor (GEF) 11* (*arhgef11*)⁹³. Arhgef11 shows localization to the apical surface of pronephric epithelium and may have potential direct or indirect roles in coordinating the actin cytoskeleton⁹³. However, *arhgef11* morphants did not show disruption of other polarity components including Prkci at the apical surface, suggesting that abrogating Arhgef11 does not completely abolish cell polarity⁹³. The abrogation of other polarity constituents has similarly not led to wide scale nephron polarity disruption, such as seen in *crb2b* morphants⁸⁶. These findings implicate epithelial polarity as being fundamentally important for nephron function, but potentially resilient to some manipulation. The extent of this resiliency and/or redundancy between other polarity constituents is still widely unknown.

The various zebrafish pronephros epithelia also exhibit discrete segment-specific features, which have been best characterized in the PCT. A conserved feature of proximal tubules is the possession of extensive endocytic machinery that is used for bulk recovery of low molecular weight solutes. The zebrafish PCT shows reabsorption of traceable dextran conjugates²⁵ (Figure 8(a)), and these have been shown to colocalize with the endocytic machinery proteins and the early endosomal marker, Rab4⁹⁴. Apical polarity is essential for proximal tubule endocytosis, as illustrated by the finding that the absence of Prkci in the *heart and soul* (*has*) mutant perturbs Rab4 positive endocytosis⁹⁵. The zebrafish PCT utilizes conserved receptor-mediated endocytosis machinery that is assembled during PCT formation and includes the protein encoded by the *low density lipoprotein receptor-related protein 2a* (*Lrp2a*, formerly known as *megalyn*) and its binding partners encoded by *cubilin* (*intrinsic factor-cobalamin receptor*) (*cubn*) and *disabled homolog 2* (*Drosophila*) (*dab2*)^{94,95}. *Lrp2a* and *dab2* transcripts can be detected at 20 hpf in the PCT, while *cubn* expression begins at 24 hpf^{94,95}. Loss of Lrp2a or Dab2 function leads to reabsorption failure, shown by uptake assays for 70 kDa fluorescent-dextran, and abrogates early endosome trafficking^{95,96}. These findings establish a vital role for endocytosis in proper reabsorption in the PCT pronephros epithelium.

Emergence of single- or multi-ciliated tubule cell types: Tubule epithelium differentiation also involves ciliogenesis. The cilium is a vital organelle for renal cells predominantly because cilia perform roles in mechanosensory transduction and fluid movement. Two ciliated cell types exist in a mosaic pattern in the nephron tubule: single-ciliated solute transporter (or primary ciliated) cells, and multiciliated cells (MCCs)^{79,97} (Figure 7(c)). The switch between these two cell types is Notch dependent, mediated by interactions between Notch receptors and ligands on adjacent tubule progenitors^{79,97} (Figure 5). Notch signaling regulates the expression of several pro-MCC fate genes such as the ciliogenic transcription factor *rfx2*. For example, *rfx2* is initially expressed ubiquitously throughout the tubule and duct nephron progenitors until approximately the 17-somite stage⁷⁹. Following this time, Notch signaling restricts *rfx2* transcripts in tubule cells, which leads to adoption of a transporter cell identity^{79,97}. Chemical inhibition of Notch signaling with DAPT leads to MCC expansion, while over-expression of an activated Notch1a intracellular domain (NICD1a) (under heat shock control in doubly-transgenic hsp70:UAS/Gal4:Notch1aICD) at the 6–7 somite stage abrogates MCC formation^{79,97}. This differentiation mechanism

between MCCs and transporting cells falls into the classical Notch lateral inhibition mechanism that produces a “salt and pepper” pattern of Jagged and Notch-expressing cells respectively^{79,97}.

The ciliogenesis process involves a gene regulatory network directed by the forkhead box (Fox) class of forkhead/winged helix domain containing transcription factors. Two paralogues of Foxj1 exist (*foxj1a*, *foxj1b*) in zebrafish and show expression in ciliated organs of the developing zebrafish such as the floor plate mesendoderm (neural tube/spinal cord), dorsal forerunner cells (DFC)/Kupffer’s vesicle, otic vesicle, and the pronephros^{98,99}. *foxj1a* transcripts were found to be expressed uniformly in all tubule cell types at 24 hpf, but down-regulated in proximal tubule single ciliated cells at 48 hpf, while both *foxj1a* and *foxj1b* were found in pronephros MCC cells^{98,99}. Intriguing evidence suggests that floor plate expression of Foxj1a is dependent on Hedgehog signaling⁹⁸, but that the expression of Foxj1a within the pronephros is reliant on WNT signaling as identified through transgenic enhancer bashing within putative Lef1/Tcf binding sites¹⁰⁰. Foxj1a promotes the specific activation of the motile cilia transcriptome, suggesting it is a key regulator for ciliary apparatus production. Foxj1a has been shown to be both necessary and sufficient to activate motile cilium-specific components including: *dnah9* (*dynein*, *axonemal*, *heavy polypeptide 9*), *ctn2* (*centrin2*), *efhc1* (*an EF hand containing protein*), *rxf2*, and *zgc:101797* (formerly known as *tektin1*)^{99,100}. However, knockdown of *foxj1a* does not completely impair MCC cilia motility, potentially because of redundancy with *foxj1b* within the pronephros⁹⁹.

Relationship of the pronephros with the corpuscle of Stannius—The pronephros is developmentally associated with an endocrine gland known as the corpuscle of Stannius (CS) (Figure 2). The CS is found in teleost and holostean fishes, where it functions in calcium homeostasis¹⁰¹. The zebrafish CS arises from the pronephros tubule field, in the boundary region where the DE and DL tubule segments meet²⁶. The CS precursors are first detectable as scattered cells at 24 hpf that express the genes *stanniocalcin 1*, *like* (*stc1l*, formerly known as *stc1*), the *spalt* family transcriptional regulator *sal-like 1a* (*Drosophila*) (*sall1a*), and *gata3*^{26,102,103}. Later, the CS cells emerge dorsally and aggregate to form a bilateral pair of clusters by 48 hpf^{26,102,103}. It remains unclear if CS cells correspond to a particular renal cell type in other vertebrates. One speculation is that CS cells might be evolutionarily related to the macula densa, a group of cells in the mammalian distal tubule that sense solute concentrations in the glomerular filtrate and regulate blood pressure⁵.

Pronephric duct morphogenesis and formation of the cloaca—The very last section of the pronephros, the pronephric duct (PD), must fuse to the exterior environment for the proper excretion of waste. The common opening that drains both the gut and pronephros is termed the cloaca. The PD will undergo morphogenesis and join ventral tail cells to form the cloaca during late somitogenesis. Accordingly, the cloaca is a composite of two-cell types—the IM-derived PD and epidermis. Cell cohesion among the PD population is essential for this process, and depletion of *cadherin 17*, *LI cadherin* (*liver-intestine*) (*cdh17*) leads to a lack of proper fusion of the PD at the cloaca¹⁰⁴.

The development and proper morphogenesis of the cloaca is also dependent on BMP signaling, which is essential for the specification of ventro-ectodermal cells^{105–107} (Figure 5). BMP signaling is complex and uses a host of downstream factors to mitigate its effects. In accordance with this signaling complexity, the morphogenesis of the cloaca is reliant on a number of cellular processes including apoptosis, migration, and cellular rearrangement^{105,106}. Labeling of ventral epidermal cells with GFP (*msxb-gfp*) has demonstrated a single large vacuolated cell migrating to the presumptive site of the cloaca. This epidermal cell fuses to the ventral most aspect of the epidermis to form the cloaca, and has been posited to form the pore in the epidermis via cell death¹⁰⁵. Terminal kidney cells

migrate almost simultaneously to reach this ventral pore¹⁰⁵. Following this cellular migration, terminal kidney cells adopt a tall-columnar morphology as the cloaca is formed¹⁰⁵. A time-course of ventral kidney terminus remodeling, as assayed by a riboprobe against *gata3*, reveals the kidney terminus reaching the ventral limit of the tail at the 22 somite stage and creation of the external opening at the 25 somite stage¹⁰⁵. *gata3* expression is restricted away from the distal most tip of the embryo, the presumptive cloaca opening, just prior to the embryo forming a cloaca¹⁰⁵. A plausible explanation for the reduction in *gata3* expression hinges on potential migration of epidermal cells over the kidney terminus prior to cloaca formation¹⁰⁵. However, the possibility has not been excluded that terminal kidney cells die off, and thereby allow the epidermal cells the ventral terminal space for the formation of the cloaca. Moreover, cell death is known to be occurring in the area of presumptive cloaca formation in wild-type embryos^{105,106}. In transgenic embryos with inducible inhibition of BMP signaling no cell death occurs and the cloaca fails to form¹⁰⁵.

Ciliary proteins known as nephrocystins, which form complexes at the base of the cilium and have roles in regulating cilium function, have also been implicated in cloaca morphogenesis^{108,109}. For example, knockdown of *nephronophthisis 4* (*nphp4*, formerly known as nephrocystin-4) morphants also fail to elicit a cell death response during cloaca formation¹⁰⁸. *nphp4* morphants, despite having an intact BMP signaling cascade, fail to develop a proper cloaca in a minority of embryos and subsequently produces bulbous cystic fluid accumulation at the presumed site of cloaca opening¹⁰⁸. Interestingly, double knockdown of *nphp4* and *par-6 partitioning defective 6 homolog gamma B* (*C. elegans*) (*pard6gb*) showed a marked increase in the number of affected embryos with an aberrant cloaca formation¹⁰⁸. Time lapse imaging of these double morphants show a defective migratory process with an absence of cellular protrusions that are typically directed towards the epidermis in wild-type embryos¹⁰⁸. As has been noted through different genetic aberrations, interfering with aspects of cloacal morphogenesis produce dire consequences including the inability of the organism to excrete waste, ultimately leading to mortality.

Onset of pronephros function and tubule morphogenesis

Essential role of fluid flow: Fluid flow is now known to play a very important part during the morphogenesis of the zebrafish pronephros. As mentioned, the glomerulus begins to function by approximately 48 hpf, shortly following the invasion of endothelial cells and the development of the vasculature interface²⁵. Glomerular filtration was assessed by injecting the circulatory system of zebrafish embryos with fluorescently-conjugated dextran moieties, then assaying whether the dextran can be visualized in the nephron lumen and proximal epithelial cells^{25,110}. Embryos injected with dextran between 33–36 hpf did not exhibit luminal fluorescence, while 48 hpf embryos exhibited fluorescence in both the nephron lumen and in brightly stained membrane vesicles²⁵ (as seen in Figure 8(a)). Once filtrate escapes the glomerulus it is propelled through the nephron by motile cilia¹¹⁰. Cilia-driven fluid flow is required for proper development of the pronephros, and mediates the tubular injury response as well¹¹⁰ (discussed in the next section). Defects in cilia formation or function leads to kidney cyst formation, in which pockets of fluid accumulate in the nephron and perturb ongoing renal activity^{7,8} (See Sidebar Box 1).

Sidebar 1

Cilia and Cystic Kidney Disease

The cilium is an apically extruding organelle ensheathed by the plasma membrane with a microtubule axoneme at its center. Eukaryotic cilia are assembled and maintained via an intraflagellar transport process where protein components are conveyed in both anterograde and retrograde fashion along the axoneme. With more than 2600 entries in

the cilium proteome database, evidence for ciliary complexity is readily transparent (www.ciliaproteome.org). Cilia dysfunction is associated with numerous and diverse forms of kidney disease. The abrogation of fluid flow and mechanosensory transduction, two primary functions of the cilium, appear to be substantial factors in these renal disease states. This presents the cilium as a quintessential cornerstone for normal kidney function. A large body of work has shown that the zebrafish is a superb system to model cystic kidney disease pathophysiology⁷. For example, mutations in polycystins (PC1 and PC2), both of which localize to the cilium, lead to autosomal dominant polycystic kidney disease (ADPKD) the most common genetically inherited disorder in humans. Zebrafish mutants of PC1 and PC2 display renal cysts and recapitulate many aspects of the PKD disease state. Thus, the zebrafish model offers the opportunity to study cilia biology and dysfunction *in vivo*. Such work has continued to shed new insights into ciliogenesis as well as other cellular mechanisms that include the planar cell polarity (PCP) pathway, which has also been implicated in renal disease progression^{120, 121}.

Tubule morphogenesis and collective cell migration: Following the onset of fluid flow at 48 hpf, gross changes in the anatomical locations and sizes of the tubule segments transpire over the next several days of development (Figure 8). The gene expression domains for distal tubule segments expand, and come to occupy more rostral positions²⁶. These changes are accompanied by morphogenesis of the PCT, which progressively coils, becoming more rostrally condensed and convoluted²⁶ (Figure 8(b)). The PCT segments first take on a distinctive morphology that resembles a ‘coat hanger’ or hairpin-like appearance, then form loops and coils (Figure 8(b)). In addition, the PST domain expands in size and shifts rostrally along the trunk²⁶.

Interestingly, live time-lapse imaging has captured distal cell populations migrating in an anterior directed manner between 28.5 hpf and 84 hpf¹¹¹. One explanation of this phenomenon proposes that distal cells migrate as a collective unit in an upstream manner against the flow of filtrate and towards the glomerulus¹¹¹. These tubule cells are hypothesized to retain their epithelial identity (including apical-basal polarity and junctional complexes) and migrate collectively in this anterior-directed manner¹¹¹. Collective cell migration was observed in the distal tubule even in the presence of camptothecin, a cell proliferation-blocking agent, suggesting that cell movements are not solely driven by nephron growth¹¹¹. However, these data do not exclude the possibility that cell proliferation occurs during pronephros morphogenesis, but rather suggests that its absence over short time intervals does not entirely abolish morphogenesis processes. Indeed, it is very likely that cell proliferation eventually contributes to pronephros growth during the first week of life.

Deficiencies in calcium and Polycystins, which are cation channel proteins, have revealed potential interactions between these constituents and collective cell migration¹¹². Calcium signaling during earlier development (~16 hpf) plays significant roles in convergence-related cell positioning and morphology within the early developing pronephric anlagen¹⁸. Furthermore, one isoform of a conserved calmodulin-dependent protein kinase, known as *calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma 1 (camk2g1)*, was identified as being present and localized to the zebrafish kidney at 30 hpf¹¹². Camk2g1 activation was found to be dependent on calcium levels regulated by the TRP ion channel PKD2 encoded by the *polycystic kidney disease 2 (pkd2)* gene¹¹². In both PKD2 and Camk2g1 morphant embryos, PCT convolutions are diminished and have been attributed to a failure in anterior cell migration¹¹².

There is also evidence that collective cell migration of pronephric epithelium requires fluid flow¹¹². Both distal nephron obstruction and perturbed glomerular filtration were reported to

abolish proximal tubule convolution and collective cell migration¹¹¹. These findings suggest strongly that fluid flow influences tubule morphogenesis. With this in mind, it is noteworthy that the PKD2 and Camk2g1 morphant embryos also show defects in cilia number and length¹¹². These defects may perturb fluid flow and secondarily lead to defects in collective cell migration. Further delineation of fluid flow dynamics and collective cell migration is needed to tease apart the interrelatedness of these two processes.

Dynamic tubular response to damage in the pronephros, and models of acute kidney injury

—Over the first week of life, the pronephros exhibits the capacity for injury response that is mediated by the cilium mechanosensory organelle, where an increased pronephric lumen diameter increases ciliary beat frequency⁹⁹. This increase in ciliary beat frequency and reciprocal increases in fluid flow correlates tightly with an increase expression of *foxj1a* in the distended tubule segment⁹⁹. Moreover, *foxj1a* is required to maintain maximal increases in MCC beat frequencies as *foxj1a* morphants exhibited a diminished cilium beat frequency response after injury⁹⁹. Both mechanical obstructions of the nephron and cystic kidney mutants like *fleer* (*flr*) show increased *foxj1a* expression⁹⁹. The onset of increased *foxj1a* expression was noted after cystic distension and interestingly *foxj1a* expression was only increased in tubule segments proximal to sites of mechanical obstruction, where epithelial distension had occurred⁹⁹. This has led to the proposal that an epithelial stretch-based mechanism is activating *foxj1a* expression⁹⁹.

Intriguing evidence for further dynamicism within the nephron has been documented in the context of tubular damage, where cell ablation was employed to selectively destroy proximal tubule epithelial cells¹¹³. Epithelial gaps were observed after ablation and confirmed by whole mount *in situ* hybridization for epithelial cell markers. When embryos with a damaged nephron were raised, intact nephron tubules were detected after several days, suggestive of epithelial regeneration. Currently, it is unclear where these cells originated from, but one possibility would be the collective cell migration and proliferation of nearby epithelium. These observations suggest that the zebrafish will provide an excellent model to study renal epithelial regeneration¹¹³.

The embryonic pronephros has also been used to examine acute kidney injury from the nephrotoxin gentamicin, an aminoglycoside antibiotic. Injection of gentamicin into the cardiac venous sinus¹¹⁴ or cardinal vein^{115,116} causes a dosage-dependent decline in glomerular filtration, tubular cell death, shedding of epithelial cells from the basement membrane, tubule distention, and gross edema formation that is fatal^{114–116}. The injection of the drug cisplatin causes similar nephron toxicity, likewise leading to tubular shedding and edema¹¹⁴. Both of these nephrotoxin models show typical features of human acute kidney injury following gentamicin or cisplatin exposure. Thus, embryonic exposure to gentamicin or cisplatin provides a model for studying the events of renal injury and can be used to screen for pharmaceuticals that prevent, attenuate or aggravate nephron damage in clinical settings.

More recently, an inducible model of pronephros injury was reported, in which a transgenic system was created to cause injury to podocytes¹¹⁷. Transgenic zebrafish were engineered to express bacterial nitroreductase (NTR) under the control of the podocyte-specific promoter of zebrafish *nphs2*¹¹⁷. In both embryos and adult fish, podocyte injury was triggered when the prodrug metronidazole was provided¹¹⁷. This study suggests that the NTR-metronidazole system will be tractable to create inducible tubular damage models as well. As cell injury correlates to metronidazole dosage, the NTR system may provide a method to titrate damage in particular pronephros tubule segment(s) in the absence of renal failure, as observed in gentamicin based models, and for researchers without access to laser ablation technologies.

Colonization by hematopoietic stem cells—By approximately 5 days post fertilization (dpf), the zebrafish pronephros becomes the residence for hematopoietic stem cells (HSCs)^{118,119}. The HSCs migrate to the glomerular region, and can be detected in migratory paths along the tubules^{118–120}. During the remainder of juvenile and adult life, HSCs reside in association with the nephron tubules of zebrafish. This is a feature that makes the pronephros and mesonephros unique from many higher vertebrates such as mammals, where the bone marrow serves as the adult HSC niche and site of hematopoiesis.

DEVELOPMENT OF THE ZEBRAFISH MESONEPHROS

Mesonephros development commences after ~2 weeks of life, upon larval growth to approximately 4–5 mm in length, and results in the addition of nephrons to the preexisting pronephros tubules (Figure 9(a)). New nephrons are generated from individual mesenchymal cells associated with the pronephric tubule^{121,122}. The initial nephrons arise in a conserved spatial position, lying ventral to the sixth somite and just posterior to the swim bladder^{121,122}. The generation of new nephrons in the mesonephros is marked by analogous expression of several developmental genes. For example, the individual mesenchymal cells show reporter expression in a transgenic line where green fluorescent protein (GFP) is produced under the control of the *lhx1a* promoter¹²². The *lhx1a*:eGFP⁺ cells are migratory, and were observed to move along the pronephros tubules when the larvae reach a length of 4 mm (corresponding to approximately 10 dpf)¹²². These mesenchymal *lhx1a*:eGFP⁺ cells aggregated, then expanded to form a cluster¹²² (Figure 9(b)). This cluster lengthened to form a tubule-like structure located in close association with the existing pronephros¹²² (Figure 9(b)). As the new tubule lengthened, regional gene expression changes were detected with additional transgenic reporters¹²² (Figure 9(b)). Cells at the junction between the new nephron and the pronephros showed expression of *lhx1a*:eGFP, a distal tubule region was marked by *pax8*:DsRed, and *wt1b*:eGFP marked the proximal tubule and glomerulus regions¹²² (Figure 9(b)). Elongated tubules were present by the time the juvenile fish grew to 5 mm in length, or approximately 13 dpf¹²². These findings suggest that nephrogenesis is extremely rapid, occurring over the course of just a few days.

With continued juvenile growth, greater numbers of new nephron aggregates coalesce and plumb into pre-existing renal tubules to form renal vesicles that subsequently become new nephrons^{121,122}. Over time, new renal vesicles are observed in association with the pronephros in locales rostral to the swim bladder and along the very distal regions of the tubule^{121,122}. The continual nephrogenesis during larval life generates a mesonephros comprised of several hundred nephron units (Figure 9(a)). Based on the observation that mesonephros tubules form drainage connections with the pronephros nephrons, it seems likely that the pronephros components persist over time, and gradually undergo a shift towards a collecting duct-like functionality. In fact, the adult mesonephros contains parallel tracks of two central collecting ducts, and it is enticing to speculate that these ducts represent remnants (albeit likely remodeled) of the original pronephros. Fate mapping studies are needed to definitively ascertain the relationship between pronephros and mesonephros components.

The adult mesonephros occupies a position on the dorsal wall of the body cavity (Figure 10). The mesonephros has distinctive regions: a head region located in a rostral position, followed by a saddle and tail regions (Figure 10(a)). Nephrons in the mesonephros exhibit a similar segmentation pattern as the pronephros. For example, they show restricted gene expression of PCT, PST, DE, and DL markers^{121–124}. Interestingly, branched DE and DL segments are detected in the adult kidney, suggestive of the notion that nephrons can be added to many positions of existing nephrons¹²². Interspersed among the nephrons is a

dense stroma comprised of HSCs, the many blood cell derivatives, and renal progenitors (Figure 10(a, b)).

The absolute number of mesonephric nephrons has a linear relationship to the animal's body size^{121–122}. While nephron density is variable along the length of the mesonephros, the density of nephrons along segments of the mesonephros is very consistent between different adult zebrafish¹²¹. This facilitates the subdivision of the adult zebrafish mesonephros into four regions based on nephron density: anterior nephron-dense region (ANDR), medial nephron-sparse region (MNSR), medial nephron-dense region (MNSR), and posterior nephron-sparse region (PNSR)¹²¹. This segmental nephron density suggests some level of control over the spatial arrangement of newly developing nephrons. However, any molecular evidence or insights into such a mechanism is lacking.

The adult mesonephros, like the pronephros, is also associated with an interrenal gland component. Embedded within the respective left and right lobes of the head kidney are bilateral interrenal glands⁶⁶ (Figure 10(a)). They are positioned ventrally, and have been identified in whole mount *in situ* hybridization analysis based on expression of the steroidogenic gene *cytochrome P450, subfamily XIA, polypeptide 1* (*cyp11a1*, formerly known as *side-chain cleavage enzyme*, (*scc*)). Fate mapping analysis is needed to establish the origin(s) of the adult interrenals, and to clarify their relationship(s) to the single embryonic interrenal cluster.

NEONEPHROGENESIS IN THE ADULT MESONEPHROS

During adulthood, zebrafish continue to exhibit body growth. The mesonephros shows ongoing addition of new nephrons, and is also resilient to damage incurred by existing nephrons, showing the capacity to regenerate nephron epithelial cells after injury^{121,122}. In addition, renal progenitor cells that reside throughout the stroma of the mesonephros can produce entirely new nephrons after injury, as shown by studies exploring the effects of gentamicin in adult zebrafish^{121,122} (Figure 10). The renal progenitor cells express several transcription factors, including *pax2a*, *pax8*, *wt1b*, and *lhx1a*, suggesting a number of parallels to the IM pronephros precursors and the mesenchymal precursors that generate the mesonephros during larval stages^{121,122}.

Using cell sorting and transplantation techniques, these adult zebrafish renal progenitor cells have been isolated and their lineage potential characterized using *lhx1a* and *wt1b* transgenics¹²². *lhx1a*⁺ renal cell aggregates can be transplanted into adult recipients, where their progeny drive formation of new nephrons in sites distinct from the site of transplantation¹²². Furthermore the new nephrons that are generated show functional integration into the recipient kidneys¹²². These groundbreaking experiments provide compelling evidence that there are renal progenitors within the *lhx1a*⁺ cell aggregate capable of *de novo* nephron formation, or neonephrogenesis. Interestingly, transplantation of donor *lhx1a*⁺/*wt1b*⁺ cells into a conditioned renal compromised recipient did not produce engrafted nephrons¹²². This finding suggests that changes in *wt1b* expression indicate an alteration in renal progenitor potential¹²².

Further examination of the renal progenitor population, as denoted by *lhx1a* expression, uncovered an interesting caveat to neonephrogenesis in zebrafish. Single *lhx1a*⁺ cells were repeatedly shown to be unable to generate new nephrons in conditioned renal compromised individuals¹²². This suggests that the potential for neonephrogenesis may be dependent on a 'community effect' within the aggregate of *lhx1a*⁺ cells, and that in its absence, the ability to form new nephrons is lost¹²². Consistent with these findings, laser ablation studies have demonstrated that normal mesonephric nephron formation is a cell autonomous process whereby ablation of one *lhx1a*⁺ aggregate has no effect on neighboring aggregates¹²². Thus,

while the *lhx1a*⁺ aggregate is necessary to initiate nephrogenesis within the mesonephros, the stemness properties, cellular interactions, and ultrastructure between different cells within the aggregate remain unknown.

Conclusion

Studies of zebrafish kidney development have provided a strong foundation for the continued exploration of renal ontogeny. Moving forward, several major questions in nephrology can be addressed using future studies in the zebrafish. First, the conserved segmental anatomy of the zebrafish pronephros provides a genetic system to identify segmentation pathways. Insights into nephrogenesis may provide useful information to understand the cause of congenital kidney defects and perhaps suggest methods of treatment. The ability of pronephros nephrons to respond to damage lends the opportunity to study acute nephron injury, which remains a poorly understood and devastating condition in humans. Finally, understanding the pathways that regulate renal progenitors during mesonephros development and adult homeostasis may lead to the identification of their mammalian counterpart, or unveil how to stimulate regenerative pathways in adults. The attributes of the zebrafish model thus provide a wealth of exciting opportunities for researchers to make progress on understanding these phenomena in the years to come.

Acknowledgments

FUNDING

The work of the Wingert laboratory is supported by the NIH-NIDDK (grant number K01DK083512), an NIH Director's New Innovator award (grant number DP2OD008470), a March of Dimes Basil O'Connor Starter Scholar award, funds from the University of Notre Dame College of Science and Department of Biological Sciences, and a generous gift to the University of Notre Dame from Elizabeth and Michael Gallagher on behalf of the Gallagher Family to foster stem cell research. We thank Paul Kroeger, Yue Li, and Kristen McCampbell for their critical review of the manuscript, and KM for providing panels for parts of Figure 8 and 10. We thank all the members of our lab for stimulating discussions and support, and the staff of the Center for Zebrafish Research at Notre Dame for their dedicated work in maintaining our zebrafish facility.

References

1. Reilly, RF.; Bulger, RE.; Kriz, W. Structural-functional relationships in the kidney. In: Schrier, RW., editor. *Diseases of the Kidney and Urinary Tract*. Philadelphia, PA: Lippincott Williams & Wilkins; 2007. p. 2-53.
2. Dressler GR. The cellular basis of kidney development. *Annu Rev Cell Dev Biol*. 2006; 22:509–529. [PubMed: 16822174]
3. Costantini F, Kopan R. Patterning a complex organ: branching morphogenesis and nephron segmentation in kidney development. *Dev Cell*. 2010; 18:698–712. [PubMed: 20493806]
4. Lieschke GJ, Currie PD. Animal models of human disease: zebrafish swim into view. *Nat Rev Genet*. 2007; 8:353–367. [PubMed: 17440532]
5. Wingert RA, Davidson AJ. The zebrafish pronephros: a model to study nephron segmentation. *Kid Int*. 2008; 73:1120–1127.
6. Drummond IA, Davidson AJ. Zebrafish kidney development. *Methods Cell Biol*. 2010; 100:233–260. [PubMed: 21111220]
7. Swanhart LM, Cosentino CC, Diep CQ, Davidson AJ, de Caestecker M, Hukriede NA. Zebrafish kidney development: basic science to translational research. *Birth Defects Res C Embryo Today*. 2011; 93:141–156. [PubMed: 21671354]
8. Ebarasi L, Oddsson A, Hultenby K, Betsholtz C, Tryggvason K. Zebrafish: a model system for the study of vertebrate renal development, function, and pathophysiology. *Curr Opin Nephrol Hypertens*. 2011; 20:416–424. [PubMed: 21519251]

9. Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. *Dev Dyn*. 1995; 203:253–310. [PubMed: 8589427]
10. Davidson AJ. Uncharted waters: nephrogenesis and renal regeneration in fish and mammals. *Pediatr Nephrol*. 2011; 26:1435–1443. [PubMed: 21336813]
11. Kimmel CB, Warga RM, Schilling TF. Origin and organization of the zebrafish fate map. *Development*. 1990; 108:581–594. [PubMed: 2387237]
12. Warga RM, Nüsslein-Volhard C. Origin and development of the zebrafish endoderm. *Development*. 1999; 126:827–838. [PubMed: 9895329]
13. Krauss S, Johansen T, Korzh V, Fjose A. Expression of the zebrafish paired box gene *pax[zf-b]* during early neurogenesis. *Development*. 1991; 113:1193–1206. [PubMed: 1811936]
14. Picker A, Scholpp S, Böhli H, Takeda H, Brand M. A novel positive transcriptional feedback loop in midbrain-hindbrain boundary development is revealed through analysis of the zebrafish *pax2. 1* promoter in transgenic lines. *Development*. 2002; 129:3227–3239. [PubMed: 12070097]
15. Toyama R, Dawid IB. *lim6*, a novel LIM homeobox gene in the zebrafish: comparison of its expression pattern with *lim1*. *Dev Dyn*. 1997; 209:406–417. [PubMed: 9264264]
16. Swanhart LM, Takahashi N, Jackson RL, Gibson GA, Watkins SC, Dawid IB, Hukriede NA. Characterization of an *lhx1a* transgenic reporter in zebrafish. *Int J Dev Biol*. 2010; 54:731–736. [PubMed: 20209443]
17. Kanki JP, Ho RK. The development of the posterior body in the zebrafish. *Development*. 1997; 124:881–893. [PubMed: 9043069]
18. Lam PY, Webb SE, Leclerc C, Moreau M, Miller AL. Inhibition of stored Ca²⁺ release disrupts convergence-related cell movements in the lateral intermediate mesoderm resulting in abnormal positioning and morphology of the pronephric anlagen in intact zebrafish embryos. *Develop Growth Differ*. 2009; 51:429–442.
19. Davidson AJ, Zon LI. The ‘definitive’ (and ‘primitive’) guide to zebrafish hematopoiesis. *Oncogene*. 2004; 23:7233–7246. [PubMed: 15378083]
20. Gering M, Rodaway ARF, Göttgens B, Patient RK, Green AR. The *SCL* gene specifies haemangioblast development from early mesoderm. *EMBO J*. 1998; 17:4029–4045. [PubMed: 9670018]
21. Davidson AJ, Ernst P, Wang Y, Dekens MP, Kingsley PD, Palis J, Korsmeyer SJ, Daley GQ, Zon LI. *cdx4* mutants fail to specify blood progenitors and can be rescued by multiple *hox* genes. *Nature*. 2003; 425:300–306. [PubMed: 13679919]
22. Dietrich HW 3rd, Kieran MW, Chan FY, Barone LM, Yee K, Rundstadler JA, Pratt S, Ransom D, Zon LI. Intraembryonic hematopoietic cell migration during vertebrate development. *Proc Natl Acad Sci USA*. 92:10713–10717.
23. Liao EC, Paw BH, Oates AC, Pratt SJ, Postlethwait JH, Zon LI. SCL/Tal-1 transcription factor acts downstream of *cloche* to specify hematopoietic and vascular progenitors in zebrafish. *Genes Dev*. 1998; 12:621–626. [PubMed: 9499398]
24. Pfeiffer PL, Gerster T, Lun K, Brand M, Busslinger M. Characterization of three novel members of the zebrafish *Pax2/5/8* family and dependency of *Pax5* and *Pax8* expression on the *Pax2. 1 (noi)* function. *Development*. 1998; 125:3063–3074. [PubMed: 9671580]
25. Drummond IA, Majumdar A, Hentschel H, Elger M, Solnica-Krezel L, Schier AF, Neuhauss SC, Stemple DL, Zwartkruis F, Rangini Z, et al. Early development of the zebrafish pronephros and analysis of mutations affecting pronephric function. *Development*. 1998; 125:4655–4667. [PubMed: 9806915]
26. Wingert RA, Selleck R, Yu J, Song HD, Chen Z, Song A, Zhou Y, Thisse B, Thisse C, McMahon AP, et al. The *cdx* genes and retinoic acid control the positioning and segmentation of the zebrafish pronephros. *PLoS Genet*. 2007; 3:1922–1938. [PubMed: 17953490]
27. Wingert RA, Davidson AJ. Zebrafish nephrogenesis involves dynamic spatiotemporal expression changes in renal progenitors and essential signals from retinoic acid and *irx3b*. *Dev Dyn*. 2011; 240:2011–2027. [PubMed: 21761484]
28. Serluca FC, Fishman MC. Pre-pattern in the pronephric kidney field of zebrafish. *Development*. 2001; 128:2233–2241. [PubMed: 11493543]

29. Bollig F, Mehringer R, Perner B, Hartung C, Schäfer M, Scharl M, Volff JN, Winkler C, Englert C. Identification and comparative expression analysis of a second *wt1* gene in zebrafish. *Dev Dyn*. 2006; 235:554–561. [PubMed: 16292775]
30. Nichane M, Van Campenhout C, Pendevel H, Voz ML, Bellefroid EJ. The Na^+/PO_4 cotransporter *SLC20a1* gene labels distinct restricted subdomains of the developing pronephros in *Xenopus* and zebrafish embryos. *Gene Expr Patterns*. 2006; 6:667–672. [PubMed: 16531124]
31. Mullins MC, Hammerschmidt M, Kane DA, Odenthal J, Brand M, van Eeden FJM, Furutani-Seiki M, Granato M, Haffter P, Heisenbert CP, et al. Genes establishing dorsoventral pattern formation in the zebrafish embryo: the ventral specifying genes. *Development*. 1995; 123:81–93. [PubMed: 9007231]
32. Kishimoto Y, Lee KH, Hammerschmidt M, Schulte-Merker S. The molecular nature of zebrafish *swirl*: BMP2 function is essential during early dorsoventral patterning. *Development*. 1997; 124:4457–4466. [PubMed: 9409664]
33. Nguyen VH, Schmid B, Trout J, Connors SA, Ekker M, Mullins MC. Ventral and lateral regions of the zebrafish gastrula, including the neural crest progenitors, are established by a *bmp2b/swirl* pathway of genes. *Dev Biol*. 1998; 199:93–110. [PubMed: 9676195]
34. Schmid B, Fürthauer M, Connors SA, Trout J, Thisse B, Thisse C, Mullins MC. Equivalent genetic roles for *bmp7/snailhouse* and *bmp2b/swirl* in dorsoventral pattern formation. *Development*. 2000; 127:957–967. [PubMed: 10662635]
35. Bauer H, Lele Z, Rauch GJ, Geisler R, Hammerschmidt M. The type I serine/threonine kinase receptor *Alk8/Lost-a-fin* is required for *Bmp2b/7* signal transduction during dorsoventral patterning of the zebrafish embryo. *Development*. 2001; 128:849–858. [PubMed: 11222140]
36. Mintzer KA, Lee MA, Runke G, Trout J, Whitman M, Mullins MC. *lost-a-fin* encodes a type I BMP receptor, *Alk8*, acting maternally and zygotically in dorsoventral pattern formation. *Development*. 2001; 128:859–869. [PubMed: 11222141]
37. Payne TL, Postlethwait JH, Yelick PC. Functional characterization and genetic mapping of *alk8*. *Mech Dev*. 2001; 100:275–289. [PubMed: 11165484]
38. Hild M, Dick A, Rauch GJ, Meier A, Bouwmeester T, Haffter P, Hammerschmidt M. The *smad5* mutation *somitabun* blocks *Bmp2b* signaling during early dorsoventral patterning of the zebrafish embryo. *Development*. 1999; 126:2149–2159. [PubMed: 10207140]
39. Miller-Bertoglio VE, Fisher S, Sanchez A, Mullins MC, Halpern ME. Differential regulation of *chordin* expression domains in mutant zebrafish. *Dev Biol*. 1997; 192:537–550. [PubMed: 9441687]
40. Schulte-Merker S, Lee KJ, McMahon AP, Hammerschmidt M. The zebrafish organizer requires *chordin*. *Nature*. 1997; 387:862–863. [PubMed: 9202118]
41. Hammerschmidt M, Pelegri F, Mullins MC, Kane DA, van Eeden FJM, Granato M, Brand M, Furutani-Seiki M, Haffter P, Heisenberg CP, et al. two genes regulating dorsal development in the zebrafish embryo *dino* and *mercedes*. *Development*. 1995; 123:95–102. [PubMed: 9007232]
42. Schulte-Merker S, van Eeden FJ, Halpern ME, Kimmel CB, Nüsslein-Volhard C. *no tail (ntl)* is the zebrafish homologue of the mouse *T(Brachyury)* gene. *Development*. 1994; 120:1009–1015. [PubMed: 7600949]
43. Morley RH, Lachani K, Keefe D, Gilchrist MJ, Flicek P, Smith JC, Wardle FC. A gene regulatory network directed by zebrafish *No tail* accounts for its roles in mesoderm formation. *Proc Natl Acad Sci*. 2009; 106:3829–3834. [PubMed: 19225104]
44. Kimmel CB, Kane DA, Walker C, Warga RM, Rothman MB. A mutation that changes cell movement and cell fate in the zebrafish embryo. *Nature*. 1989; 337:358–362. [PubMed: 2911386]
45. Ho RK, Kane DA. Cell-autonomous action of zebrafish *spt-1* mutation in specific mesodermal precursors. *Nature*. 1990; 348:728–730. [PubMed: 2259382]
46. Amacher SL, Draper BW, Summers BR, Kimmel CB. The zebrafish T-box genes *no tail* and *spadetail* are required for development of trunk and tail mesoderm and medial floor plate. *Development*. 2002; 129:3311–3323. [PubMed: 12091302]
47. Shimizu T, Bae YK, Muraoka O, Hibi M. Interaction of Wnt and *caudal*-related genes in zebrafish posterior body formation. *Dev Biol*. 2005; 279:125–141. [PubMed: 15708563]

48. Davidson AJ, Zon LI. The *caudal*-related homeobox genes *cdx1a* and *cdx4* act redundantly to regulate *hox* gene expression and the formation of putative hematopoietic stem cells during zebrafish embryogenesis. *Dev Biol.* 2006; 292:506–518. [PubMed: 16457800]
49. Shimizu T, Bae YK, Hibi M. Cdx-Hox code controls competence for responding to Fgfs and retinoic acid in zebrafish neural tissue. *Development.* 2006; 133:4709–4719. [PubMed: 17079270]
50. Young T, Deschamps J. Hox, Cdx, and anteroposterior patterning in the mouse embryo. *Curr Top Dev Biol.* 2009; 88:235–255. [PubMed: 19651307]
51. Tena JJ, Neto A, de la Calle-Mustienes E, Bras-Pereira C, Casares F, Gomez-Skarmeta JL. *Odd-skipped* genes encode repressors that control kidney development. *Dev Biol.* 2007; 301:518–531. [PubMed: 17011543]
52. Mudumana SP, Hentschel D, Liu Y, Vasilyev A, Drummond IA. *odd skipped related1* reveals a novel role for endoderm regulating kidney versus vascular cell fate. *Development.* 2008; 135:3355–3367. [PubMed: 18787069]
53. Lyons JP, Miller RK, Zhou X, Weidinger G, Deroo T, Denayer T, Park JI, Ji H, Hong JY, Li A, et al. Requirement of Wnt/ -catenin signaling in pronephric kidney development. *Mech Dev.* 2009; 126:142–159. [PubMed: 19100832]
54. Duester G. Retinoic acid synthesis and signaling during early organogenesis. *Cell.* 2008; 134:921–931. [PubMed: 18805086]
55. Grandel H, Lun K, Rauch GJ, Rhinn M, Piotrowski T, Houart C, Sordino P, Küchler AM, Schulte-Merker S, Geisler R, et al. Retinoic acid signaling in the zebrafish embryo is necessary during pre-segmentation stages to pattern the anterior-posterior axis of the CNS and to induce a pectoral fin bud. *Development.* 2002; 129:2851–2865. [PubMed: 12050134]
56. Begemann G, Schilling TF, Rauch GJ, Geisler R, Ingham PW. The zebrafish *neckless* mutation reveals a requirement for *raldh2* in mesodermal signals that pattern the hindbrain. *Development.* 2001; 128:3081–3094. [PubMed: 11688558]
57. Perz-Edwards A, Hardison NL, Linney E. Retinoic acid-mediated gene expression in transgenic zebrafish. *Dev Biol.* 2001; 229:89–101. [PubMed: 11133156]
58. deGroh ED, Swanhart LM, Cosentino CC, Jackson RL, Dai W, Kitchens CA, Day BW, Smithgall TE, Hukriede NA. Inhibition of histone deacetylase expands the renal progenitor cell population. *J Am Soc Nephrol.* 2010; 21:794–802. [PubMed: 20378823]
59. Majumdar A, Lun K, Brand M, Drummond IA. Zebrafish *no isthmus* reveals a role for *pax2. 1* in tubule differentiation and patterning events in the pronephric primordia. *Development.* 2000; 127(10):2089–98. [PubMed: 10769233]
60. Bedell VM, Person AD, Larson JD, McLoon A, Balciunas D, Clark KJ, Neff KI, Nelson KE, Bill BR, Schimmenti LA, et al. The lineage-specific gene *ponzr1* is essential for zebrafish pronephric and pharyngeal arch development. *Development.* 2012; 139(4):793–804. [PubMed: 22274699]
61. Reggiani L, Raciti D, Airik R, Kispert A, Brändli AW. The prepattern transcription factor *Irx3* directs nephron segment identity. *Genes Dev.* 2007; 21:2358–2370. [PubMed: 17875669]
62. Quaggin SE, Kreidberg JA. Development of the renal glomerulus: good neighbors and good fences. *Development.* 2008; 135:609–620. [PubMed: 18184729]
63. Topczewska JM, Topczewski J, Solnica-Krezel L, Hogan BL. Sequence and expression of zebrafish *foxc1a* and *foxc1b*, encoding conserved forkehead/winged helix transcription factors. *Mech Dev.* 2001; 100(2):343–347. [PubMed: 11165495]
64. 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(2):318–330. [PubMed: 21871448]
65. Kramer-Zucker AG, Wiessner S, Jensen AM, Drummond IA. Organization of the pronephric filtration apparatus in zebrafish requires Nephrin, Podocin, and the FERM domain protein Mosaic eyes. *Dev Biol.* 2005; 285(2):316–29. [PubMed: 16102746]
66. Hsu HJ, Lin G, Chung BC. Parallel early development of zebrafish interrenal glands and pronephros: differential control by *wt1* and *ff1b*. *Development.* 2003; 130:2107–2116. [PubMed: 12668625]
67. Liu YW. Interrenal organogenesis in the zebrafish model. *Organogenesis.* 2007; 3:44–48. [PubMed: 19279699]

68. Perner B, Englert C, Bollig F. The Wilms tumor genes *wt1a* and *wt1b* control different steps during the formation of the zebrafish pronephros. *Dev Biol.* 2007; 309(1):87–96. [PubMed: 17651719]
69. Bollig F, Perner B, Besenbeck B, Köthe S, Ebert C, Taudien S, Englert C. A highly conserved retinoic acid responsive element controls *wt1a* expression in the zebrafish pronephros. *Development.* 2009; 136(17):2883–2892. [PubMed: 19666820]
70. McLaughlin KA, Ronces MS, Mercola M. Notch regulates cell fate in the developing pronephros. *Dev Biol.* 2000; 227:567–580. [PubMed: 11071775]
71. McCright B, Gao X, Shen L, Lozier J, Lan Y, Maguire M, Herzlinger D, Weinmaster G, Jiang RL, Gridley T. Defects in development of the kidney, heart and eye vasculature in mice homozygous for a hypomorphic *Notch2* mutation. *Development.* 2001; 128:491–502. [PubMed: 11171333]
72. Wang P, Pereira FA, Beasley D, Zheng H. Presenilins are required for the formation of comma- and S-shaped bodies during nephrogenesis. *Development.* 2003; 130:5019–5029. [PubMed: 12930775]
73. Cheng HT, Miner JH, Lin M, Tansey MG, Roth K, Kopan R. Gamma-secretase activity is dispensible for mesenchyme-to-epithelium transition but required for podocyte and proximal tubule formation in developing mouse kidney. *Development.* 2003; 130:5031–5042. [PubMed: 12952904]
74. Cheng HT, Kim M, Valerius MT, Surendran K, Schuster-Gossler K, Gossler A, McMahon AP, Kopan R. *Notch2*, but not *Notch1*, is required for proximal fate acquisition in the mammalian nephron. *Development.* 2007; 134:801–811. [PubMed: 17229764]
75. White JT, Zhang B, Cerqueira DM, Tran U, Wessely O. Notch signaling, *wt1* and *foxc2* are key regulators of the podocyte gene regulatory network in *Xenopus*. *Development.* 2010; 137:1863–1873. [PubMed: 20431116]
76. Takemoto M, He L, Norlin J, Patrakka J, Xiao Z, Petrova T, Bondjers C, Asp J, Wallgard E, Sun Y, et al. Large-scale identification of genes implicated in kidney glomerulus development and function. *EMBO J.* 2006; 25:1160–1174. [PubMed: 16498405]
77. Smithers L, Haddon C, Jiang YJ, Lewis J. Sequence and embryonic expression of *deltaC* in the zebrafish. *Mech Dev.* 2000; 90:119–123. [PubMed: 10585570]
78. Zecchin E, Conigliaro A, Tiso N, Argenton F, Bortolussi M. Expression analysis of *jagged* genes in zebrafish embryos. *Dev Dyn.* 2005; 233:638–645. [PubMed: 15830385]
79. Ma M, Jiang YJ. Jagged2a-Notch signaling mediates cell fate choice in the zebrafish pronephric duct. *PLoS Genet.* 2007; 3(1):e18. [PubMed: 17257056]
80. Sieger D, Tautz D, Gajewski M. The role of *Suppressor of Hairless* in Notch mediated signaling during zebrafish somitogenesis. *Mech Dev.* 2003; 120:1083–1094. [PubMed: 14550536]
81. Echeverri K, Oates AC. Coordination of symmetric cyclic gene expression during somitogenesis by *Suppressor of Hairless* involves regulation of retinoic acid metabolism. *Dev Biol.* 2007; 301:388–403. [PubMed: 17098223]
82. Serluca FC, Drummond IA, Fishman MC. Endothelial signaling in kidney morphogenesis: a role for hemodynamic forces. *Curr Biol.* 2002; 12:492–497. [PubMed: 11909536]
83. Majumdar A, Drummond IA. The zebrafish *floating head* mutant demonstrates podocytes play an important role in directing glomerular differentiation. *Dev Biol.* 2000; 222:147–157. [PubMed: 10885753]
84. Majumdar A, Drummond IA. Podocyte differentiation in the absence of endothelial cells as revealed in the zebrafish avascular mutant, *cloche*. *Dev Genet.* 1999; 24(3–4):220–229. [PubMed: 10322630]
85. Müller T, Rumpel E, Hradetzky S, Bollig F, Wegner H, Blumenthal A, Greinacher A, Endlich K, Endlich N. Non-muscle myosin IIA is required for development of the zebrafish glomerulus. *Kid Int.* 2011; 80:1055–1063.
86. Ebarasi L, He L, Hultenby K, Takemoto M, Betsholtz C, Tryggvason K, Majumdar A. A reverse genetic screen in the zebrafish identifies *crb2b* as a regulator of the glomerular filtration barrier. *Dev Biol.* 2009; 334(1):1–9. [PubMed: 19393641]

87. Hsu YC, Willoughby JJ, Christensen AK, Jensen AM. Mosaic eyes is a novel component of the Crumbs complex and negatively regulates photoreceptor size. *Development*. 2006; 133:4849–4859. [PubMed: 17092952]
88. Ashworth S, Teng B, Kaufeld J, Miller E, Tossidou I, Englert C, Bollig F, Staggs L, Roberts IS, Park JK, et al. Cofilin-1 inactivation leads to proteinuria—studies in zebrafish, mice and humans. *PLoS One*. 2010; 5(9):e12626. [PubMed: 20838616]
89. Sekine Y, Nishibori Y, Akimoto Y, Kudo A, Ito N, Fukuhara D, Kurayama R, Higashihara E, Babu E, Kanai Y, et al. Amino acid transporter LAT3 is required for podocyte development and function. *J Am Soc Nephrol*. 2009; 20:1586–1596. [PubMed: 19443642]
90. Raschperger E, Neve EPA, Wernerson A, Hultenby K, Pettersson RF, Majumdar A. The coxsackie and adenovirus (CAR) is required for renal epithelial differentiation within the zebrafish pronephros. *Dev Biol*. 2008; 313:455–464. [PubMed: 18062954]
91. Nishibori Y, Katayama K, Parikka M, Oddsson A, Nukui M, Hultenby K, Wernerson A, He B, Ebarasi L, Raschperger E, et al. Glcc1 deficiency leads to proteinuria. *J Am Soc Nephrol*. 2011; 22:2037–2046. [PubMed: 21949092]
92. Horne-Badovinac S, Lin D, Waldron S, Schwarz M, Mbamalu G, Pawson T, Jan Y, Stainier DY, Abdelilah-Seyfried S. Positional cloning of heart and soul reveals multiple roles for PKC lambda in zebrafish organogenesis. *Curr Biol*. 2001; 11:1492–502. [PubMed: 11591316]
93. Panizzi JR, Jessen JR, Drummond IA, Solnica-Krezel L. New functions for a vertebrate Rho guanine nucleotide exchange factor in ciliated epithelia. *Development*. 2007; 134(5):921–931. [PubMed: 17267448]
94. McCarthy RA, Barth JL, Chintalapudi MR, Knaak C, Argraves WS. Megalin functions as an endocytic sonic hedgehog receptor. *J Biol Chem*. 2002 Jul 12; 277(28):25660–25667. [PubMed: 11964399]
95. Anzenberger U, Bit-Avragim N, Rohr S, Rudolph F, Dehmel B, Willnow TE, Abdelilah-Seyfried S. Elucidation of megalin/LRP2-dependent endocytic transport processes in the larval zebrafish pronephros. *J Cell Sci*. 2006; 119:2127–2137. [PubMed: 16638803]
96. Kur E, Christa A, Veth KN, Gajera CR, Andrade-Navarro MA, Zhang J, Willer JR, Gregg RG, Abdelilah-Seyfried S, Bachmann S, et al. Loss of Lrp2 in zebrafish disrupts pronephric tubular clearance but not forebrain development. *Dev Dyn*. 2011; 240:1567–1577. [PubMed: 21455927]
97. Liu Y, Pathak N, Kramer-Zucker A, Drummond IA. Notch signaling controls the differentiation of transporting epithelia and multiciliated cells in the zebrafish pronephros. *Development*. 2006; 134:1111–22. [PubMed: 17287248]
98. Yu X, Ng CP, Habacher H, Roy S. Foxj1 transcription factors are master regulators of the motile ciliogenic program. *Nat Genet*. 2008; 40(12):1445–1453. [PubMed: 19011630]
99. Hellman NE, Liu Y, Merkel E, Austin C, Le Corre S, Beier DR, Sun Z, Sharma N, Yoder BK, Drummond IA. The zebrafish *foxj1a* transcription factor regulates cilia function in response to injury and epithelial stretch. *Proc Natl Acad Sci USA*. 2010; 107(43):18499–18504. [PubMed: 20937855]
100. Caron A, Xu X, Lin X. Wnt/ -catenin signaling directly regulates Foxj1 expression and ciliogenesis in zebrafish Kupffer's vesicle. *Development*. 2012; 139(3):514–524. [PubMed: 22190638]
101. Kaneko T, Hasegawa S, Hirano T. Embryonic origin and development of the corpuscles of Stannius in chum salmon (*Oncorhynchus keta*). *Cell Tissue Res*. 1992; 268:65–70. [PubMed: 1499053]
102. Camp E, Hope R, Kortschak D, Cox TC, Lardelli M. Expression of three *spalt (sal)* gene homologues in zebrafish embryos. *Dev Genes Evol*. 2003; 213:35–43. [PubMed: 12590351]
103. Tseng DY, Chou MY, Tseng YC, Hsiao CD, Huang CJ, Kaneko T, Hwang PP. Effects of stannicalcin-1 on calcium uptake in zebrafish (*Danio rerio*) embryo. *Am J Physiol Regul Integr Comp Physiol*. 2008; 296:R549–R557. [PubMed: 19073903]
104. Horsfield J, Ramachandran A, Reuter K, LaVallie E, Collins-Racie L, Crosier K, Crosier P. Cadherin-17 is required to maintain pronephric duct integrity during zebrafish development. *Mech Dev*. 2002; 115:15–26. [PubMed: 12049763]

105. Pyati UJ, Cooper MS, Davidson AJ, Nechiporuk A, Kimelman D. Sustained Bmp signaling is essential for cloaca development in zebrafish. *Development*. 2006; 133(11):2275–2284. [PubMed: 16672335]
106. Pyati UJ, Webb AE, Kimelman D. Transgenic zebrafish reveal stage-specific roles for Bmp signaling in ventral and posterior mesoderm development. *Development*. 2005; 132(10):2333–2343. [PubMed: 15829520]
107. Stickney HL, Imai Y, Draper B, Moens C, Talbot WS. Zebrafish *bmp4* functions during late gastrulation to specify ventroposterior fates. *Dev Biol*. 2007; 310:71–84. [PubMed: 17727832]
108. Slanchev K, Pütz M, Schmitt A, Kramer-Zucker A, Walz G. Nephrocystin-4 is required for pronephric duct-dependent cloaca formation in zebrafish. *Hum Mol Genet*. 2011; 20(16):3119–3128. [PubMed: 21596840]
109. Burcklé C, Gaudé HM, Vesque C, Silbermann F, Salomon R, Jeanpierre C, Antignac C, Saunier S, Schneider-Maunoury S. Control of the Wnt pathways by nephrocystin-4 is required for morphogenesis of the zebrafish pronephros. *Hum Mol Genet*. 2011; 20(13):2611–2627. [PubMed: 21498478]
110. Kramer-Zucker AG, Olale F, Haycraft CJ, Yoder BK, Schier AF, Drummond IA. Cilia-driven fluid flow in the zebrafish pronephros, brain and Kupffer's vesicle is required for normal organogenesis. *Development*. 2005; 132(8):1907–1921. [PubMed: 15790966]
111. Vasilyev A, Liu Y, Mudumana S, Mangos S, Lam PY, Majumdar A, Zhao J, Poon KL, Kondrychyn I, Korzh V, et al. Collective cell migration drives morphogenesis of the kidney nephron. *PLoS Biol*. 2009; 7(1):e9. [PubMed: 19127979]
112. Rothschild SC, Francescato L, Drummond IA, Tombes RM. CaMK-II is a PKD2 target that promotes pronephric kidney development and stabilizes cilia. *Development*. 2011; 138(16):3387–3397. [PubMed: 21752935]
113. Johnson CS, Holzemer NF, Wingert RA. Laser ablation of the zebrafish pronephros to study renal epithelial regeneration. *JoVE*. 2011; 5410.3791/2845
114. Hentschel DM, Park KM, Cilenti L, Zervos AS, Drummond IA, Bonventre JV. Acute renal failure in zebrafish: a novel system to study a complex disease. *Am J Physiol Renal Physiol*. 204, 288:F923–929.
115. Cosentino CC, Roman BL, Drummond IA, Hukriede NA. Intravenous microinjections of zebrafish larvae to study acute kidney injury. *JoVE*. 2010; 4210.3791/2079
116. Rider SA, Tucker CS, del-Pozo J, Rose KN, MacRae CA, Bailey MA, Mullins JJ. Techniques for the *in vivo* assessment of cardio-renal function in zebrafish (*Danio rerio*) larvae. *J Physiol*. 2012; 590.8:1803–1809. [PubMed: 22331420]
117. Zhou W, Hildebrandt F. Inducible podocyte injury and proteinuria in transgenic zebrafish. *J Am Soc Nephrol*. 2012. 23:1039–1047.
118. Murayama E, Kissa K, Zapata A, Mordelet E, Briolat V, Lin HF, Handin RI, Herbomel P. Tracing hematopoietic precursor migration to successive hematopoietic organs during zebrafish development. *Immunity*. 2006; 25:963–975. [PubMed: 17157041]
119. Jin H, Xu J, Wen Z. Migratory path of definitive hematopoietic stem/progenitor cells during zebrafish development. *Blood*. 2007; 109:5208–5214. [PubMed: 17327398]
120. Bertrand JY, Kim AD, Ten S, Traver D. CD41+cmyb+ precursors colonize the zebrafish pronephros by a novel migration route to initiate adult hematopoiesis. *Development*. 2008; 135:1853–1862. [PubMed: 18417622]
121. Zhou W, Boucher RC, Bollig F, Englert C, Hildebrandt F. Characterization of mesonephric development and regeneration using transgenic zebrafish. *Am J Physiol Renal Physiol*. 2010; 299(5):F1040–F1047. [PubMed: 20810610]
122. Diep CQ, Ma D, Deo RC, Holm TM, Naylor RW, Arora N, Wingert RA, Bollig F, Djordjevic G, Lichman B, et al. Identification of adult nephron progenitors capable of kidney regeneration in zebrafish. *Nature*. 2011; 470(7332):95–100. [PubMed: 21270795]
123. Elizondo MR, Arduini BL, Paulsen J, MacDonald EL, Sabel JL, Henion PD, Cornell RA, Parichy DM. Defective skeletogenesis with kidney stone formation in dwarf zebrafish mutant for *tpm7*. *Curr Biol*. 2005; 15:667–671. [PubMed: 15823540]

124. Gerlach GF, Schrader LN, Wingert RA. Dissection of the adult zebrafish kidney. *JoVE*. 2011; 5410.3791/2839
125. Hostetter CL, Sullivan-Brown JL, Burdine RD. Zebrafish pronephros: a model for understanding cystic kidney disease. *Dev Dyn*. 2003; 228(3):514–522. [PubMed: 14579389]
126. Borovina A, Superina S, Voskas D, Ciruna B. Vangl2 directs the posterior tilting and asymmetric localization of motile primary cilia. *Nat Cell Biol*. 2010; 12(4):407–412. [PubMed: 20305649]
127. Cao Y, Park A, Sun Z. Intraflagellar transport proteins are essential for cilia formation and for planar cell polarity. *J Am Soc Nephrol*. 2010; 21(8):1326–1333. [PubMed: 20576807]

Figure 1. Timeline of renal development in the zebrafish

(Top) Colored bar denotes major developmental events, as marked by somite (s) number or days post fertilization (dpf). (Bottom) Black bars denote approximate time period for the various events of pronephros and mesonephros formation.

Figure 2. The zebrafish pronephros arises from the IM

(A) Tail-bud stage embryo with intermediate mesoderm (IM) (blue) situated between the paraxial mesoderm (PM) (grey) and lateral plate mesoderm (LPM) (white). (B) The 5 somite stage embryo exhibits several cell fields in close proximity to the renal progenitor cell field: domains of *tal1*⁺ cells that are thought to give rise to angioblasts, a *tal1*/*gata1a* co-expressing domain that is thought to give rise to primitive blood cells, and the field of renal progenitors which likely co-express *pax2a*, *pax8*, and *lhx1a*. (C) The 28 somite stage embryo exhibits a pronephros comprised of two parallel nephrons that consist of numerous segment domains, known as the podocytes (P) (dark green), neck (N) (light green), proximal convoluted tubule (PCT) (orange), proximal straight tubule (PST) (yellow), distal early (DE) (light blue), corpuscle of Stannius (CS) (red), distal late (DL) (dark blue), and pronephric duct (PD) (black), which drains the body via the cloaca (C) (pink).

Figure 3. Specification, axial positioning, and maintenance of the renal progenitor cell field
 (A) Ventral mesoderm fates arise upon BMP signaling during D-V axis specification. Fate map studies at the embryonic shield stage have determined the locale (blue) from which the blood, angioblasts, and pronephros cell lineages arise. Blood and angioblasts arise from the ventral-most mesoderm, with pronephros fates partially intermingled but also occupying a slightly more lateral position. (B) Mesoderm specification events that promote IM fates (blue) involve the actions of *T-box* factors, such as *ntl* and *tbx16*, which are expressed during late gastrula (epiboly) stages and throughout somitogenesis (not depicted). (C) Mesodermal patterning by the *cdx* factors is responsible for setting the axial position of the IM field (blue). Patterning events in adjacent tissues also affect pronephros progenitors, such as *osr1* activity, which leads to the maintenance of *pax2a*-expression in the rostral renal progenitor cell field through its effects in regulating endoderm and angioblast lineages.

Figure 4. Emergence of nephron progenitor subdomains

(A) The renal progenitor cell field develops from the IM directly adjacent to the field of paraxial mesoderm (PM) (dorsal view). The PM cells, which are patterned through pathways that include the activities of *ntla*, *tbx16*, and *cdx* genes, secrete retinoic acid (RA). RA secretion (purple arrows) is highest in the rostral PM during the late gastrula to early somitogenesis stages, leading to the hypothesis that the renal progenitor cell field is exposed to a gradient of RA that is highest in rostral regions adjacent to where the first few somites will form. (B–E) Gene expression subdomains in the renal progenitor cell field are as indicated: rostral (light blue), central (turquoise), and caudal (dark blue). These domains are speculated to represent major subdivisions of the nephron progenitors, though this pattern of subdomains is actually an intricate, nested pattern that is both complex and dynamic. Gene expression domains fluctuate through to the 28 somite stage, when segment boundaries can be detected based on discrete domains of solute transporter transcripts. (B) At the 5 somite stage, the rostral domain contains a subset of *wt1a*⁺ cells (area of wide blue cross-hatchings in the rostral field), that includes the podocyte progenitors. (C) By the 8 somite stage, the rostral and caudal fields have overlapping boundaries, where a region of cells co-expresses *jag2* and *mecom* (dark blue cross-hatchings). (D) By the 15 somite stage, *irx3b* expression is

detected, and has been the basis of support for the notion of a central domain identity at this timepoint. The podocyte lineage (green box) is distinct from the tubular populations, based on the specific expression of *foxc1a*, *hey1*, *lhx1a*, *mafba*, and *wt1b* in cells located adjacent to somite 3; a broader region still expresses *wt1a*, likely including interrenal progenitors (wide blue cross-hatchings adjacent to somite 2) (See also Figure 6). (E) By the 28 somite stage, segments have emerged as abutting domains that are defined by the expression of specific solute transporter gene transcripts and/or transcription factors, though most transcription factors examined to date mark multiple segments. Nephron segment abbreviations are: podocytes (P) (dark green), neck (N) (light green), proximal convoluted tubule (PCT) (orange), proximal straight tubule (PST) (yellow), distal early (DE) (light blue), corpuscle of Stannius (CS) (red), distal late (DL) (dark blue), and pronephric duct (PD) (black), with the cloaca (C) (pink).

Figure 5. Nephron gene regulatory network

The signals and transcription factors that are thought to direct pronephros lineages are indicated; black arrows indicate positive/inductive signals and red lines indicate inhibitory roles. Nephron segment abbreviations are: podocytes (P) (dark green), neck (N) (light green), proximal convoluted tubule (PCT) (orange), proximal straight tubule (PST) (yellow), distal early (DE) (light blue), corpuscle of Stannius (CS) (red), distal late (DL) (dark blue), and pronephric duct (PD) (black), with the cloaca (C) (pink). The PST and DE segments are comprised of a mosaic pattern of two epithelial cell populations (not shown in segment bar): single-ciliated solute transporter cells and multiciliated cells (MCC). The differentiation of MCC requires Notch signaling, shown with blue arrows.

Figure 6. Glomerular and interrenal gland development

(A) Anatomical location of the podocyte and interrenal fields in the zebrafish embryo that is enlarged in panels B–E, in which nephrons are shown in a region of the embryo trunk that is just ventral to the spinal cord, notochord, and midline somites. (B) Several partially-overlapping gene expression domains define the podocyte and interrenal precursors during late somitogenesis, *wt1a* (light purple) is expressed in a broad field adjacent to somites 1–3, *wt1b* is restricted to an area of cells (dark green) adjacent to somite 3, and *nr5a1a* is expressed in scattered cells (dark purple) littered throughout the *wt1a/b* fields. (C) By the 28 somite stage, the *nr5a1a*+ cells have formed bilateral clusters just caudal to the *wt1b*+ cells (*wt1a* domain no longer indicated). (D) At 30–36 hpf, the podocytes migrate to the midline and join together, and the interrenal cells also migrate to the midline. (E) By 48 hpf, the podocytes have recruited vasculature to form a glomerulus (G), and the interrenal gland (IR) remains a single conglomerate.

Figure 7. Tubule epithelium development

(A) At 24 hpf (the 28 somite stage), the nephron tubules have undergone a mesenchymal to epithelial transition (MET), and are visible as bilateral tubule units in cross section. (B) Immunohistochemistry image of cryosection from a *cdh17::eGFP* embryo, with the right nephron tubule shown enlarged in (B'). (B') Digital zoom (6.25X) of a single nephron tubule cross section, with the nephrons visible as GFP+ cells, with tubulin (light blue) detected at the apical epithelial surface (red), and nuclei stained with DAPI (blue), 60X magnification. White bar indicates 5 microns. (C) At 48 hpf, the nephron tubule epithelium has further differentiated, and single or multiple ciliated organelles are present in cells of the PST and DE, depicted in a sagittal section schematic (note: cell number in PST domain is not representative of actual PST segment cell number); epithelium maturation coincides with the onset of glomerular filtration, and subsequent fluid flow within the tubule (blue arrow).

Figure 8. Morphogenesis of the pronephros proximal tubule

(A) The proximal convoluted tubule (PCT) exhibits receptor mediated endocytosis and shows uptake of solutes, as visualized by absorption of 40 kilodalton fluorescein isothiocyanate (FITC) dextran conjugates. (Top) A brightfield image of a 4 dpf embryo that was injected with FITC-dextran at 2 dpf, shown laterally, (middle) view of the PCT through FITC filter, and (bottom) enlarged view of a single PCT. (B) PCT coiling morphogenesis, shown in dorsal view after whole mount *in situ* hybridization to detect *slc20a1a* transcripts (stained in purple). (Top) 3 dpf embryo shows 'coat hanger' hooks of the rostral PCT, (middle) 4 dpf embryo where coiling has now occurred, and (bottom) 5 dpf embryo where the PCT has multiple loops and coils.

Figure 9. Mesonephros development: addition of nephrons to the pronephros

(A) (Top) At approximately 10–14 dpf (corresponding to ~ 4 mm in length), juvenile zebrafish larvae begin mesonephros development. indicates region of the pronephros enlarged below. (Bottom left panel) Single *lhx1a*+ mesenchymal cells first appear in association with the distal tubule near the swim bladder, in a region indicated by asterisk (green). (Bottom right panel) Over time, the progressive addition of mesonephros tubules (pink) leads to a dense collection of nephrons that plumb into the pair of pronephros nephrons (blue). (B) The process of tubulogenesis is indicated. Single *lhx1a*+ cells multiply and form aggregates in close juxtaposition to the existing pronephros tubule. Over several days, the *lhx1a*+ cells rapidly expand and generate a nephron tubule that eventually displays polarized gene expression domains of *lhx1a* (green), *pax8* (purple), and *wt1b* (pink) expressing cells.

Figure 10. The adult mesonephros

(A) (Top) Adult zebrafish with pink box indicating dorsal body wall location of the mesonephros. (Middle) Schematic dorsal view of the mesonephros, with head, trunk and tail regions; the approximate positions of the ventrally-located interrenal glands are indicated with an asterisk (*). (Bottom) Schematic of branched tubular network in the mesonephros. Branched pinwheel arrays of nephrons plumb into large collecting ducts. The interstitial stroma includes hematopoietic stem cells (HSC) (light blue), blood cells (pink; specific lineages not designated), and renal progenitors (green). The stroma may also include other mesenchymal cell type(s) yet to be identified. (B) Histological section of a paraffin-embedded adult kidney stained with hematoxylin and eosin (60X magnification). The glomerulus (G), tubule (T) and blood (b) fractions are indicated with black arrows.