Chapter 1

Renal Development and Anatomy

William L. Clapp

Knowledge of the intricate structures of the developing kidney and the adult kidney provides insight into their functions and facilitates an understanding of renal diseases. One cannot recognize what is abnormal in the kidney if one does not know what is normal. This chapter considers kidney development, both its morphogenesis and regulatory mechanisms, followed by the anatomy and function of the adult kidney. The focus is on the human kidney, but some insights largely derived from other mammals will be discussed.

Renal Development

How can a kidney of elaborate nephrons with multiple cell types develop from aggregates of primitive mesenchymal cells? It is one of science's most profound questions. Renal development is dynamic and represents a classic model for studying organogenesis. The kidney builds itself from the "adaptive self-organization" of DNA, RNA and proteins which leads to cell differentiation, intercellular interactions and construction of complex tissue compartments (1). A basic understanding of kidney development provides a framework to enhance our knowledge of congenital anomalies of the kidney and urinary tract (CAKUT), the most common cause of pediatric chronic kidney disease (2). Studies of the developing kidney will also likely yield insights into adult kidney disorders, including renal repair after injury and renal cancer. Finally, a detailed comprehension will be necessary for renal regenerative biologic studies using stem/progenitor cells, chemical compounds and decellularized matrices (scaffolds).

Embryonic Kidneys

The urogenital system is the last organ system to form and the metanephric (permanent) kidney is the last of three excretory organs to develop. The pronephros, mesonephros and metanephros form in a cranial to caudal sequence from the intermediate mesoderm, which is situated between the dorsal somites and the lateral plate mesoderm. The pronephros and mesonephros are transient embryonic structures in mammals, although their sequential development is essential for formation of the metanephros.

Silva's Diagnostic Renal Pathology 2nd edn. ed. Zhou *et al.* Published by Cambridge University Press. © Cambridge University Press 2017.

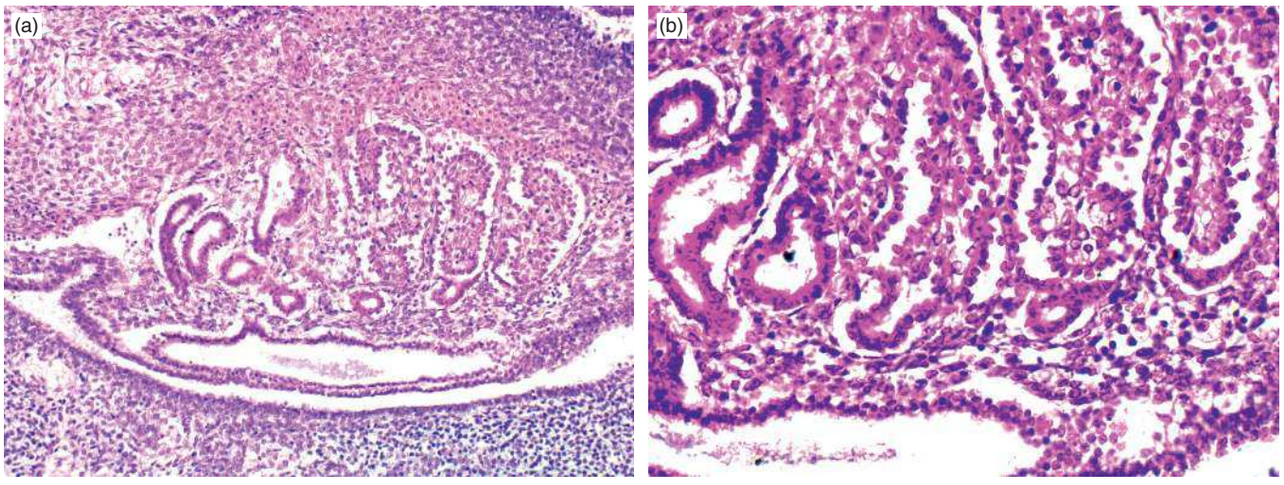

Figure 1.1 Mesonephros. (A) Light micrograph of mesonephros from a 5- to 6-week gestation human embryo showing glomeruli, tubules and portion of mesonephric duct. (H&E, × 200.) (B) Higher magnification illustrating well-formed tubules (left) and glomeruli (right) with rows of podocytes on outside of capillary loops. (H&E, × 400.)

The pronephros forms at the end of the 3rd week of gestation in the cervical region. It consists of a glomus (glomerulus-like), tubules and a duct. The pronephros is rudimentary and non-functional in humans. Although the glomus and tubules involute, the pronephric duct persists to become the mesonephric duct. The zebrafish pronephros is a valuable model to study the cell and molecular processes that are conserved in mammalian kidney development and go awry in renal diseases (3).

The mesonephros develops in the 4th week of gestation in the thoracic region. It consists of about 30 nephrons consisting of glomeruli connected to tubules, with proximal and distal segments, some of which join the mesonephric duct (*Wolffian* duct or simple nephric duct) (Figure 1.1A,B). The distal mesonephric duct communicates with the cloaca, a precursor of the urinary bladder. Although some excretory function of the human mesonephros may exist, it is believed to be transient and limited. Like the pronephros, the mesonephros degenerates. In males, some tubules develop as the efferent ducts of the epididymis and the mesonephric duct forms the duct of the epididymis, the vas deferens, and the seminal vesicle. In females, mesonephros involution leaves only vestigial structures such as the epoophoron, paroophoron and Gartner duct. The formation of the paramesonephric duct (*Mullerian* duct) is dependent on the mesonephric duct (4). The Mullerian duct degenerates in males but in females gives rise to the oviducts, uterine horns, cervix, and anterior vagina. Numerous genes involved in the formation of the metanephros are also expressed in the mesonephros, suggesting the two kidney forms share some common molecular pathways of development (5,6). Thus, the embryonic kidneys are essential for the evolvement of the metanephros and they also contribute to the development of the male and female genital systems.

Metanephros

This section deals with the development of the metanephros with a focus on its morphogenesis (7–11). Representing the permanent kidney in mammals, the metanephros forms from a mutual inductive interaction between the ureteric bud, an outgrowth from the mesonephric duct (nephric duct), and the metanephric mesenchyme, an aggregate of cells in the caudal intermediate mesoderm. Although the ureteric bud (UB) and metanephric mesenchyme both originate from the intermediate mesoderm, their respective epithelial and mesenchymal cell types provide for complex molecular signaling between each other. In the 5th week of gestation, the UB grows dorsally until it reaches the metanephric mesenchyme (Figures 1.2A,B). Upon contact, the UB undergoes branching morphogenesis to form the collecting system, which includes the renal pelvis, calyces, and collecting ducts. Induced by the UB, the metanephric mesenchyme forms early epithelial structures which differentiate into nephrons containing glomeruli, proximal and distal tubules, and Henle's loops. Thus, the cells of the collecting system and the nephrons derive from two different lineages.

Collecting System: Renal Pelvis, Calyces, and Ducts

Reaching the metanephric mesenchyme, the UB undergoes immediate branching (Figure 1.3). The branching is rapid, repetitive, and complex with simultaneous branch elongation. This intricate branching pattern establishes the arborizing architecture of the kidney. The UB branches consist of a trunk or stalk portion, which elongates, and a distal ampullary tip. The trunk of the initial UB forms the ureter. The first three to five generations of UB branches form the renal pelvis, with more branching at the poles compared to the midpolar region (Figure 1.4). Progressive dilatation and coalescence of these early branches, presumably due to urine production, forms the early pelvic–calyceal system by 12 weeks. The terminal portions of the next generation of branches remain somewhat constricted to form the major calyces. Subsequent branching forms the minor calyces which undergo significant remodeling. Each minor calyx is associated with several ampulla from which the

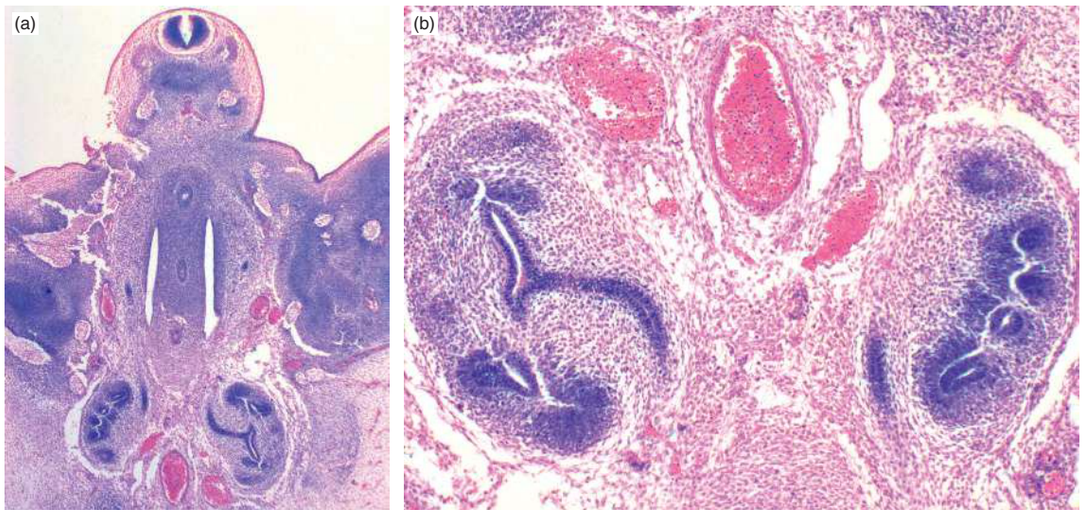

Figure 1.2 (A) Transverse section of a 6-week gestation human embryo showing paired metanephric kidneys. The abdominal aorta containing red cells is situated between the kidneys. (H&E, × 40.) (B) Higher magnification of section (Figure 1.2A) showing that the symmetrical reniform character of metanephric kidneys is established early in development. Branching of the ureteric bud is seen in the right kidney. (H&E, × 100.)

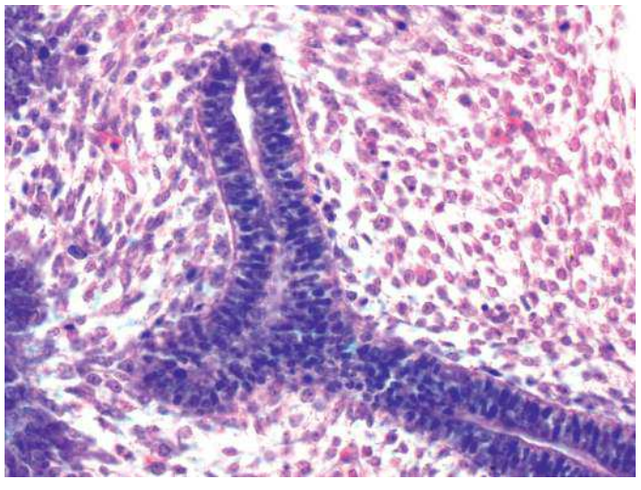

Figure 1.3 Metanephros from a 6-week human embryo. Early branching of the ureteric bud (UB) is evident. A basement membrane surrounds the ureteric epithelium. (H&E, × 400.)

papillary collecting ducts originate. By 14 weeks, the expanding renal pelvis and nephrons induced by the collecting ducts in the developing papillae compress the calyces. The papillae become conical and indent the minor calyces as they convert from a bulbous configuration to a cup-like shape (Figure 1.5). At 8 weeks, the first nephrons are induced by the action of the ampulla on the surrounding metanephric mesenchyme. For a significant time, the two basic organogenetic processes of branching morphogenesis and nephron formation occur simultaneously.

Collecting duct morphogenesis has been divided into four periods (7). In the first period, 5th–14th weeks of gestation, dichotomous branching occurs from the ampullary tips and individual nephrons remain attached to their ampullae. In an iterative bifurcation model, one of the two new ampullae retains the old nephron, whereas the other induces the formation of a new one. The second period, weeks 14–22, is characterized by the formation of arcades. Ampullae rarely branch but elongate with single tips repeatedly inducing new nephrons while carrying attached older nephrons. With new nephron formation, the connecting tubule of the older nephron merges its point of attachment away from the ampulla to the connecting tubule of the newer nephron. This process is repeated resulting in 3–7 nephrons forming around a single ampulla, joined together in an arcade by their connecting tubules. These early nephrons joined in arcades become the juxtamedullary nephrons in the inner cortex of the fully developed organ.

In the 3rd period, weeks 20–36, the ampullae advance beyond the attachment point of the arcade, toward the outer cortex. They do not branch but induce 5–7 nephrons, each of which will have a direct connection to the developing collecting duct. This direct type of nephron attachment predominates in the outer cortex of the mature kidney, whereas arcades are situated in the inner cortex (Figure 1.6). Since the nephrons remain contacted with their ampullae of origin, either directly or through arcades, the continual longitudinal growth of the collecting ducts eventuates in the attached glomeruli being positioned in the cortex. In the 4th period, weeks 36 to term, the ampullae disappear and no new nephrons form. Nephrogenesis does not normally occur beyond 36 weeks of gestation. The last nephrons formed are in the outer cortex with their glomeruli near the renal capsule.

Nephron Formation

A fairly accurate morphologic view of human nephron development has been known for a long time (Figure 1.7) (12). *Nephrogenesis* more strictly refers to the process of nephron generation, but it is often used to indicate all aspects of kidney development. Nephron formation can be divided into two

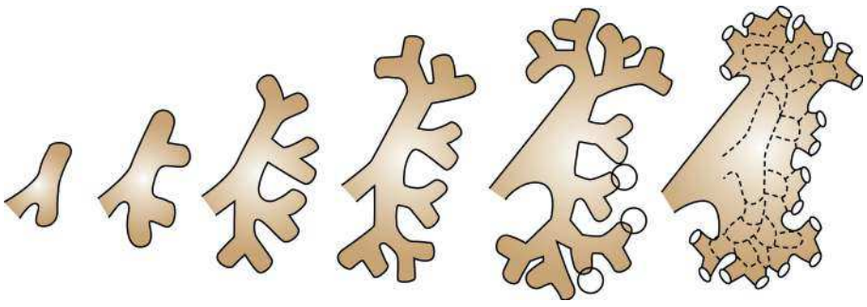

Figure 1.4 Formation of the renal pelvis. The diagram illustrates expansion and coalescence of early UB branches to form the pelvis. (Modified from Potter EL. *Normal and Abnormal Development of the Kidney*. Chicago: Year Book Medical Publishers, Inc. 1972.)

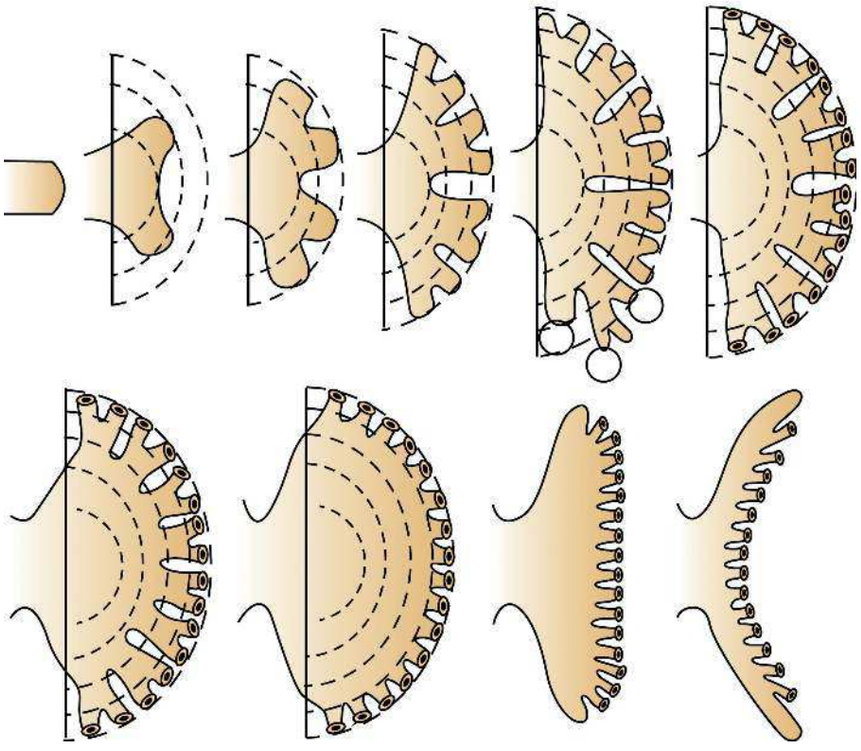

Figure 1.5 Formation of a renal calyx and papilla. The pelvis continues to expand and the peripheral zone of differentiating nephrons compress the original saccular cavity, forming the cup-like shape of the calyx and the conical form of the papilla. (Modified from Potter EL. *Normal and Abnormal Development of the Kidney*. Chicago: Year Book Medical Publishers, Inc. 1972.)

stages: induction and morphogenesis. With induction, the metanephric mesenchyme condenses around the ampullary tips in response to signals from the tips. Two types of condensates may be distinguished. The first one, called the *cap mesenchyme*, forms a layer several cells thick which closely surrounds each ampullary tip (Figure 1.8A,B). The cap mesenchyme is believed to regulate ureteral branching and contains the progenitor cells of the nephron epithelia. Later in time, another condensate, the *pretubular aggregate*, forms at the lateral edges of the ampullary tip below the cap mesenchyme. The cells of the pretubular aggregate differentiate from the cap mesenchyme and are believed committed to form elements of the nephron.

The morphogenetic stage involves several complex events. The cells of the pretubular aggregate undergo a mesenchymal-to-epithelial transition characterized by cell polarization, a surrounding basal lamina and a central cavity, at which point the structure is called a *renal vesicle*. Multipotential cells within the renal vesicle will give rise to all the epithelial cell types of the nephron. A lower vascular cleft develops representing the site where glomerular capillaries will emerge. The renal vesicle becomes a *comma-shaped* tubular structure. An upper cleft develops and the comma elongates and folds leading to an *S-shaped body* representing an early nephron form (Figure 1.9). During these stages, the upper part of the S-shaped body fuses to the same ureteric branch tip that initially induced its structure. Thus, the lumen of the early nephron (S-body) is now continuous with the early collecting system. At this stage, the S-body is already compartmentalized into three areas containing distinct cell types. The vascular cleft lies below the upper and middle limbs of the S-body and above the lower limb. Two epithelial layers actually compose the lower limb: visceral epithelium which will form podocytes and parietal epithelium which will line Bowman's capsule. The midportion (limb) will become the proximal tubule and Henle's loop. The upper limb develops into the distal convoluted tubule and fuses with the ureteric epithelium to form the connecting tubule.

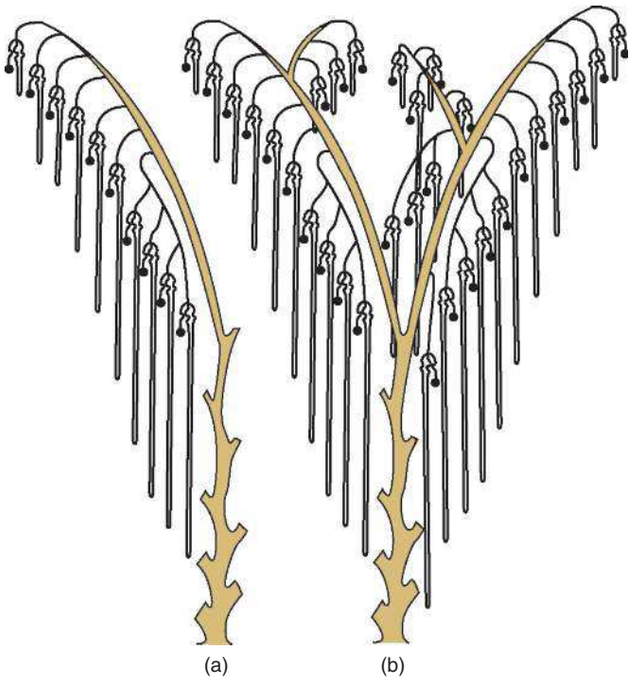

Figure 1.6 Arrangement of nephrons and collecting ducts at birth. (A) The common pattern is for each collecting duct to have a single arcade consisting of 3–5 nephrons and 5–7 nephrons individually attached. Arcades are associated with juxtamedullary nephrons in the inner cortex of the fully developed kidney, whereas the individual direct attachments predominate in the outer cortex. (B) Other patterns of attachments are possible but infrequent. (Modified from Potter EL. *Normal and Abnormal Development of the Kidney*. Chicago: Year Book Medical Publishers, Inc. 1972.)

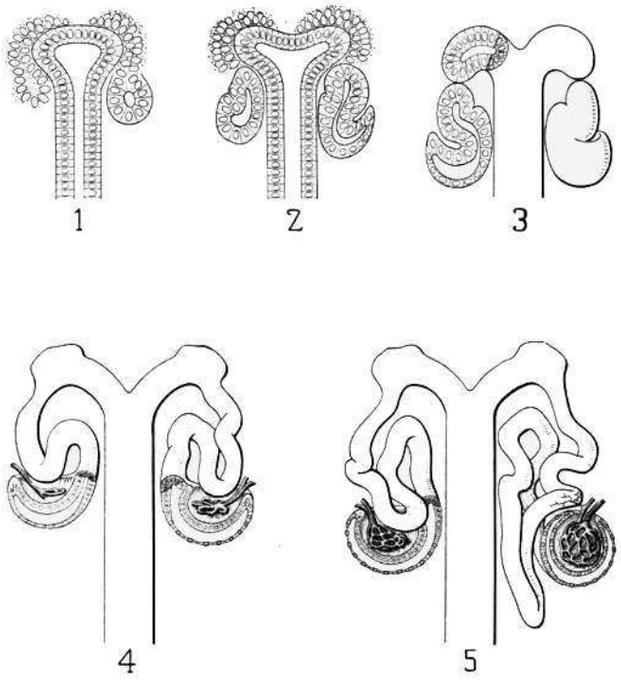

Figure 1.7 Schematic drawing of nephron formation. (1) Cap mesenchyme induced around UB ampullary tip. A renal vesicle (right) is present. (2) Comma-shaped body. (3) S-shaped body. (4) Development of early glomerular capillaries, and Bowman's capsule and tubule elongation. (5) Glomerular and tubule maturation. (From Huber GC. On the development and shape of uriniferous tubules of certain of the higher mammals. *Am J Anat* 1905;4(suppl);1–98.)

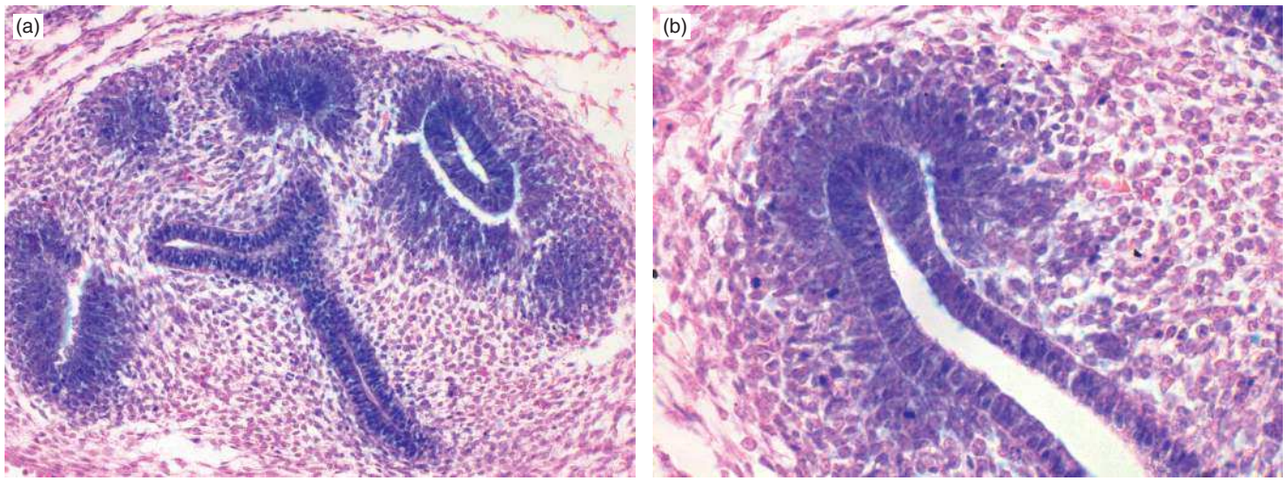

Figure 1.8 Kidney from 6-week human embryo. (A) The branching UB has induced the metanephric mesenchyme (MM) to form condensates called the cap mesenchyme (CM). The stromal mesenchyme has a loose appearance (H&E, × 200). (B) UB branch with cap mesenchyme from developing kidney at 6 weeks of gestation. The ureteral epithelium at the ampullary tip is thickened and surrounded by a cohesive collar of cap mesenchyme (H&E, × 400).

Nephron formation occurs across the developing cortex in a band called the *nephrogenic zone* (Figure 1.10A–D). Continued branching and growth of the ureteric epithelium and the incremental layout of nephrons results in a centrifugal developmental pattern in the cortex. The earliest nephrons formed are in the juxtamedullary cortex, whereas the last nephrons made are in the outer cortex. By about 36 weeks, nephrogenesis ceases and the nephrogenic zone disappears. The side-by-side processes of ureteral branching morphogenesis and nephrogenesis build the architectural organization of the kidney.

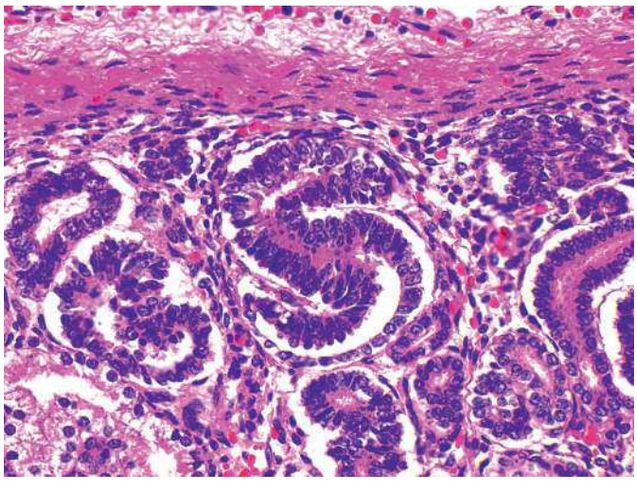

Figure 1.9 S-shaped body from developing kidney at 26 weeks of gestation. The glomerulus and its attached tubule segments will differentiate from the S-body. (H&E, × 400.)

Glomerulogenesis

Glomerular development proceeds through a sequence of structures: vesicle, comma, S-body, capillary loop, and maturing glomerulus (Figure 1.11). In the comma- and S-shaped stages, microvessels believed derived from endothelial precursors (angioblasts) can be seen within the vascular cleft. At this stage, the developing capillary endothelium contains few fenestrae but begins laying down a basement membrane. The early columnar-shaped podocytes also assemble a basement membrane which is usually thicker and more continuous. Thus, two basal laminae exist between the endothelial and podocyte layers. During the loop stage, the capillaries fill out into Bowman's capsule and the endothelial cells flatten and form numerous fenestrae. The podocytes develop an intricate cyto-architecture as they flatten, form primary elongated processes, which in turn, elaborate foot processes which interdigitate with those from adjacent podocytes. Apical junctional complexes migrate down the sides of the foot processes and convert into slit diaphragms bridging between adjacent foot processes. In the maturing stage, endothelial cells and podocytes continue to differentiate. The podocytes may have a cuboidal appearance but are near terminal differentiation and no longer divide (Figure 1.12). The dual basement membranes have fused into a single glomerular basement membrane (GBM), made mainly by the podocytes. Where foot process interdigitation is incomplete, irregular outpockets of basement membrane are found beneath podocytes, reflecting new basement membrane segments which will be spliced into the GBM. The formation of the mesangium occurs relatively later in glomerulogenesis. There remains much to be learned about it.

Vasculature

Compared to other components, little is known about the development of the renal vasculature. In general development and pathologic conditions, vessels can be formed by two processes. In vasculogenesis, inherent precursor cells differentiate into endothelia which becomes organized into vessels. In angiogenesis, new vessels arise from existing ones by sprouting and migrating. Most favor that the renal vasculature likely develops from a combination of both processes. Vasculogenesis is more prominent in early kidney development, especially in the development of glomerular capillaries. Angiogenesis involves the larger renal vessels sprouting from the renal artery in later stages.

Interstitium

The loose stromal mesenchyme containing spindle-shaped cells and surrounding the early ureteral branches and early nephrons is known as the primary interstitium ("clear cell stroma") (Figures 1.3 and 1.8A). In the nascent cortex, it is situated peripheral to the more dense cap mesenchyme and includes the renal capsule. Thus, the stroma is a distinctive cellular compartment within the nephrogenic zone. As development proceeds, a cortical stroma and a medullary stroma, each with distinct cell types, forms. There is much to be learned about renal stroma development and function.

Mechanisms of Renal Development

The making of a kidney involves the coordination of complex cellular and molecular events. Several experimental approaches and model systems have increased our understanding. They include *in vitro* organ cultures (13) and *in vivo* genetic manipulations in animals (14,15). The most powerful model has been the mouse. Several human CAKUT disorders have mutations of genes that were first discovered in the mouse. High-throughput global gene expression studies have provided insights into discrete compartments in the developing kidney that cannot otherwise be easily distinguished (16). Recent studies have pushed the limits of resolution by examining gene expression at the single-cell level in the developing kidney (17). Interestingly, some single progenitor cells of the metanephric mesenchyme have been shown to coexpress markers of both nephron epithelial and stromal lineages. Moreover, many single cells of the renal vesicle coexpress markers of both podocyte and proximal tubule lineages. These results suggest "promiscuous" multilineage gene expression in single progenitor cells during nephrogenesis primes the cells for subsequent specific lineage commitment. Such multilineage priming of progenitor cells preceding commitment to a single lineage has also been shown in the hematopoietic system. These types of studies have contributed to the Genitourinary Developmental Molecular Anatomy Project (GUDMAP), a valuable database for research (18). An understanding of lineage connections within the developing kidney has evolved (Figure 1.13) (19).

Intermediate Mesoderm

The intermediate mesoderm gives rise to the entire kidney morphogenetic program of the pronephros, mesonephros, and metanephros. The intermediate mesoderm is specified to a renal fate by bone morphogenetic protein (Bmp) and retinoic acid signals along the medial–lateral and cranial–caudal axes, respectively (20). The development of the transient kidneys (pronephros and mesonephros) and the metanephros in a progressive cranial–caudal direction is striking. Specific

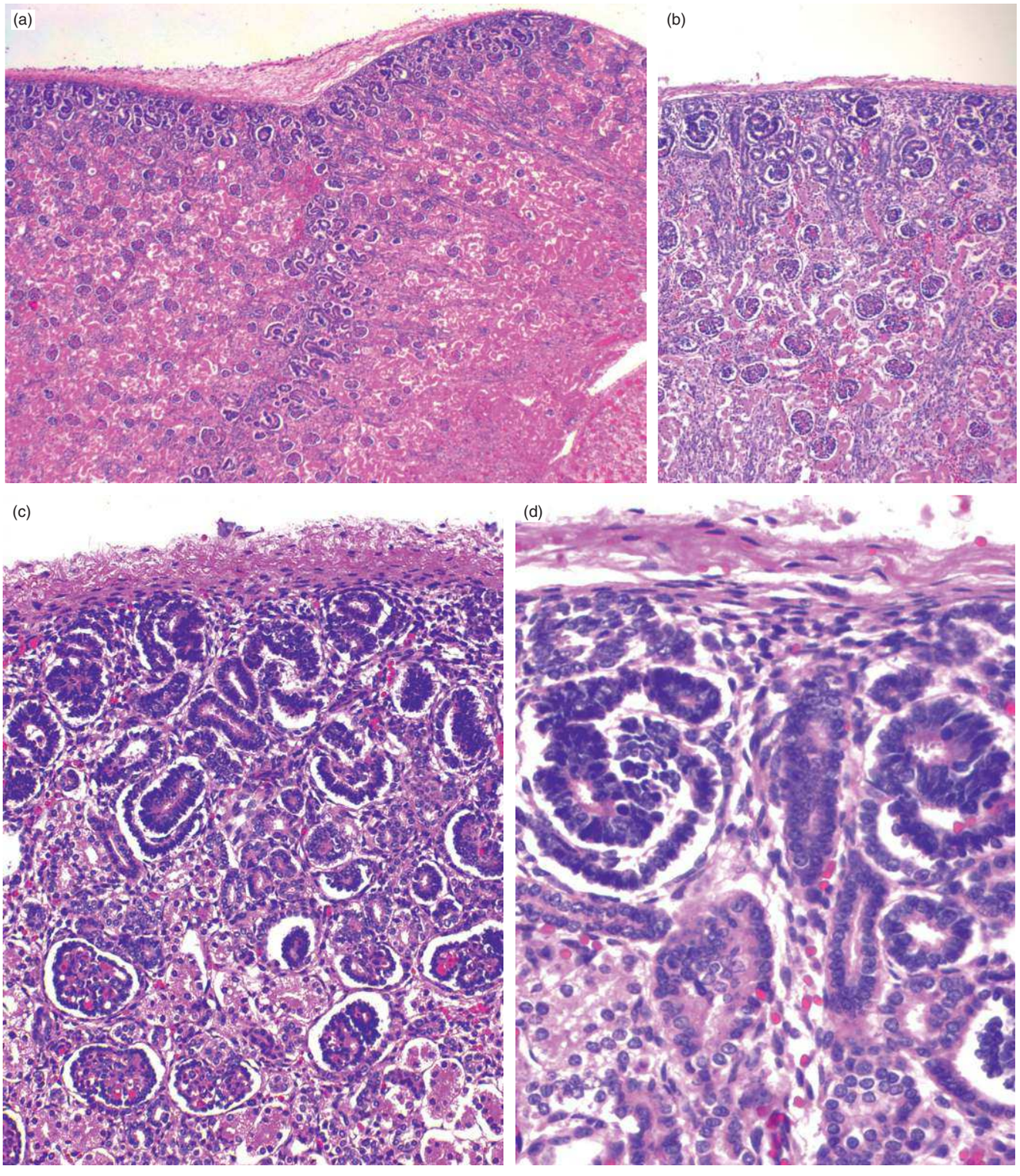

Figure 1.10 (A) Kidney at 26 weeks of gestation showing the fusion of two lobes, leaving a concavity at cortical surface. The nephrogenic zone represents the thin "blue" layer in the peripheral aspects of the lobes, both near the surface and in the midplane of the developing column of Bertin extending down between the two lobes (H&E, × 40). (B) Renal cortex at 26 weeks of gestation showing a centrifugal pattern of nephron formation and maturation. Active nephrogenesis occurs in the outer cortex, whereas nephron maturation occurs in the developing mid- and inner cortex (H&E, × 100). (C) Nephrogenic zone from developing kidney at 26 weeks of gestation. A gradient of nephron maturation is below the nephrogenic zone (H&E, × 200). (D) Higher magnification showing nephrogenic zone. An S-shaped body is present (left) (H&E, × 400).

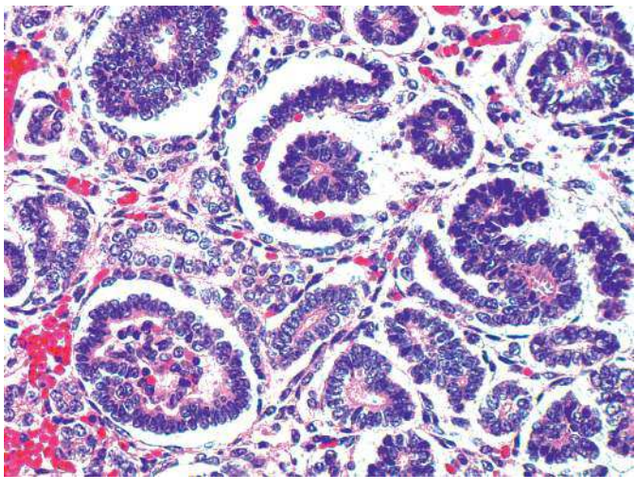

Figure 1.11 Glomerulogenesis in lower nephrogenic zone. Glomerular formation and maturation are evident. (H&E, × 400.)

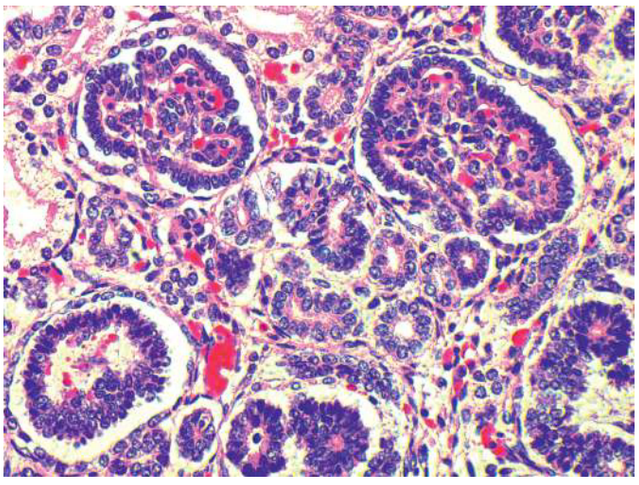

Figure 1.12 Immature glomeruli beneath the nephrogenic zone. The capillary loops contain red cells and the podocytes have a cuboidal appearance. (H&E, × 400.)

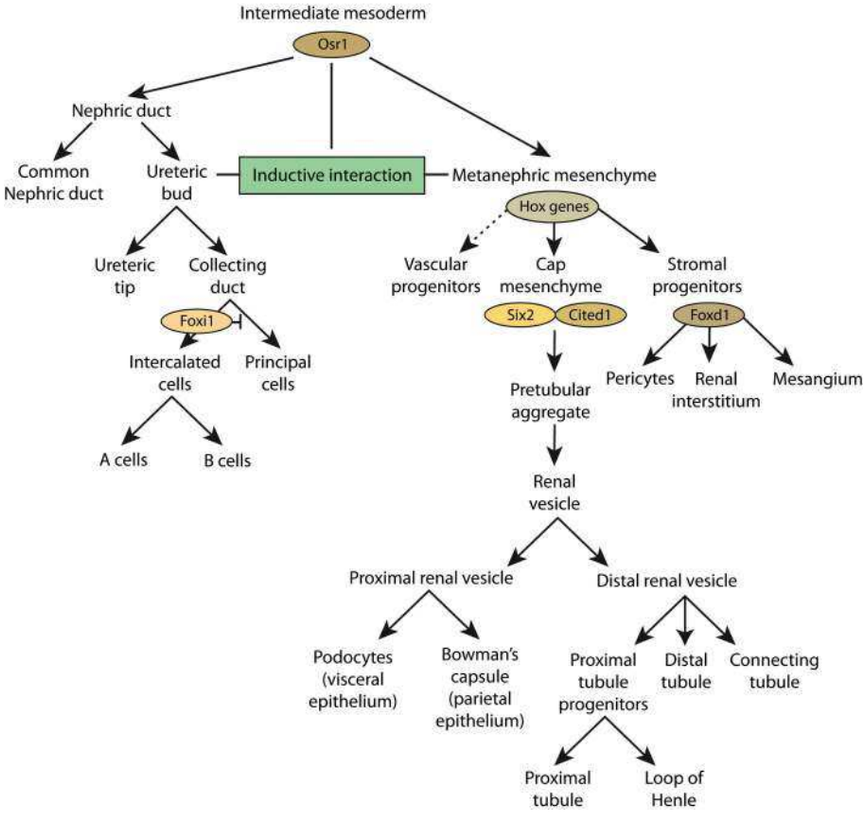

Figure 1.13 Algorithmic tree for lineage relationships within the developing kidney. The key event is the reciprocal inductive interaction (green box) between the ureteric bud and the metanephric mesenchyme. The development of the various specialized structures and cell types in the kidney depend upon this interaction. Important marker genes for the different structures and cells are indicated (in ovals). (Modified from Little MH, McMahon AP. Mammalian kidney development: principles, progress and projections. *Cold Spring Harb Perspect Biol* 2012;4:a008300.)

patterns of *Hox* gene expression establish the borders of the intermediate mesoderm that will become the metanephric mesenchyme. The earliest known genes that are induced in the intermediate mesoderm are *Osr1, Lhx1, Pax2*, and *Pax8*.

Ureteric Bud Formation

Within the intermediate mesoderm, several genes including *Pax2, Pax8, Lhx1, Gata3*, and *Ctnnb1* (β-catenin) form a regulatory network essential for nephric duct development. Signals from the metanephric mesenchyme induce budding of the UB from the nephric duct. It represents the first step in metanephric kidney development. Failure to form a UB results in renal agenesis and incorrect positioning of the UB outgrowth leads to CAKUT. *Gdnf/Ret* is the major signaling pathway (Figure 1.14) (21,22). The UB fails to form in most embryos without *Gdnf* and/or *Ret*. The glial-derived

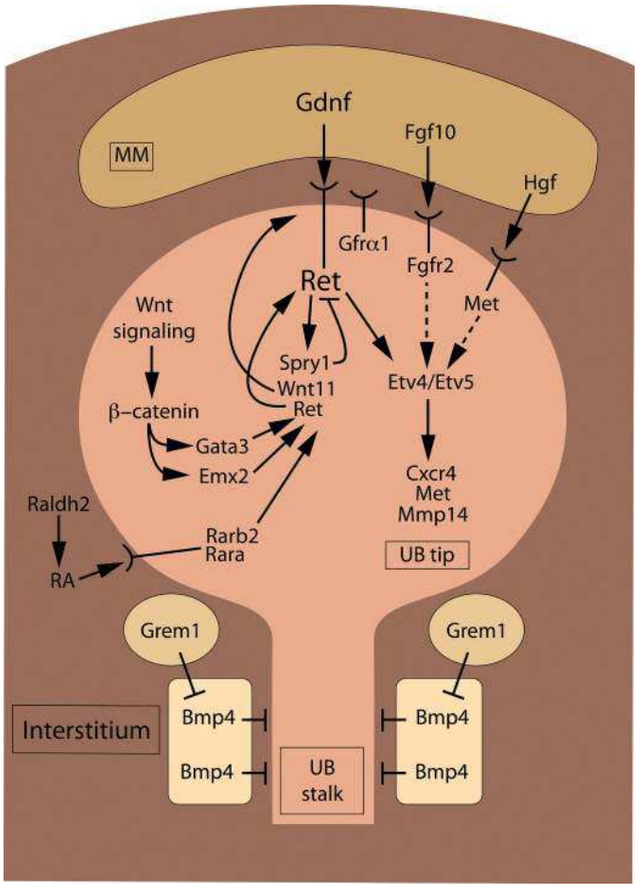

Figure 1.14 **Schematic representation of the signaling networks controlling ureteric bud formation and branching. GDNF from the metanephric mesenchyme interacting with RET in the ureteric epithelium is the key signaling pathway. A correct balance between the various activators and inhibitors of Gdnf/Ret signaling is required for normal kidney development. See the text for details. (Modified from Costantini F. GDNF/Ret signaling and branching morphogenesis. *Organogenesis* 2010;5:252–262.)**

neurotrophic factor (GDNF) secreted by the metanephric mesenchyme interacts with RET, a proto-oncogene receptor tyrosine kinase, and a coreceptor, GFRα1, both expressed in the nephric duct, to induce UB outgrowth. Before UB budding, a segment in the nephric duct thickens as a RET-rich cellular domain that will become the initial budding tip.

Several activators and inhibitors affect *Gdnf* expression in the metanephric mesenchyme. Activators include *Pax2, Eya1*, and *Hox11* paralogs, mutations of which result in defective UB formation and often renal agenesis. Inhibitors include *Sall1, Robo2, Slit2*, and *Foxc2*, mutations of which result in an opposite phenotype of multiple UBs and ureters. Although activated by *Ret*, Sprouty1 (*Spry1*) in a feedback loop negatively regulates *Ret* to limit the response to GDNF. Alternative pathways may further fine-tune the budding process. For example, *Fgf10*, expressed in the metanephric mesenchyme, can induce ureteric budding in the absence of *Gdnf/Ret* signaling. The negative regulation of UB formation by *Bmp4*, located in the interstitium surrounding the nephric duct, is in turn, suppressed by the Bmp antagonist *Grem1*. Thus, there is a complex interplay of signals controlling UB formation.

Ureteral Branching and Growth

Like UB formation, *Gdnf/Ret* signaling continues to be the key pathway in ureteral branching after the UB contacts the metanephric mesenchyme (Figure 1.14). The accumulation of RET-positive cells in the tips mediate the signaling. *Wnt11* expression also in the tips is dependent on *Gdnf/Ret* signals and reciprocally, *Gdnf* expression is dependent upon a tip *Wnt11* signal. Thus, it appears *Gdnf, Ret*, and *Wnt11* function in a positive, autoregulatory feedback loop to drive ureteral branching. Downstream RET signaling targets include the transcription factors *Etv4* and *Etv5*, which in turn activate *Cxcr4, Met*, and *Mmp4*, which play significant roles in branching.

Once thought of as a continuous, reiterative process, ureteral branching displays structural and temporal discontinuity (23,24). In early kidney development, branching is a predominant event. Later, branching slows and ureteral growth and remodeling occurs. Each ureteral branch consists of an ampullary tip and a stalk or trunk. Several branching types occur: tip-bifid branching (most common), tip-trifid branching, and stalk-lateral branching. Most of terminal branching appears to be "orthogonal" where the plane of successive bifurcations shifts by 90 degrees. It has been suggested the ureteric epithelium has its own tree-patterning system, a "self-avoidance" mechanism, such that branches form and spread out by mutal repulsion (25). Many of the genes and their encoded proteins that regulate kidney development have proven to be useful markers to study renal diseases and cancers. *Pax2* and *Gata3* are two examples (26,27). In the developing kidney, *Pax2* is expressed in the metanephric mesenchyme prior to induction, in the UB and its branches (Figure 1.15). *Pax2* expression persists in the cap mesenchyme and in the comma- and S-shaped bodies. It is transiently expressed in podocytes and eventually in parietal epithelial cells (Figure 1.16) (28). *Gata3*, positively regulated by *Pax2*, is expressed in the UB and its branches but not in early nephron forms (Figures 1.17 and 1.18) (29).

Several separate cellular processes contribute to ureteral tree morphogenesis. One is increased cell proliferation especially at the tips (30). Another involves Wnt signaling and planar cell polarity (31). Planar cell polarity refers to the organization of cells in a plane perpendicular to the apical–basal cellular axis, which in a renal duct is the plane parallel to the basement membrane along the longitudinal axis. Genes in this pathway, especially *Wnt9b*, are involved in regulating convergent extension, that is the lengthening and narrowing of the collecting ducts (32). A multicellular rosette mechanism of cell intercalation mechanism was found to control the convergent extension (33). Disruption of planar cell polarity has been documented in some models of polycystic kidney disease associated with shortened, dilated collecting ducts. A remarkable cell behavior associated with cell division has been observed in the tips of ureteral branches (34). The following is what is called "mitosis-associated cell dispersal": tip cells about to divide project out and undergo mitosis in the tubular lumen while still attached to the underlying basement

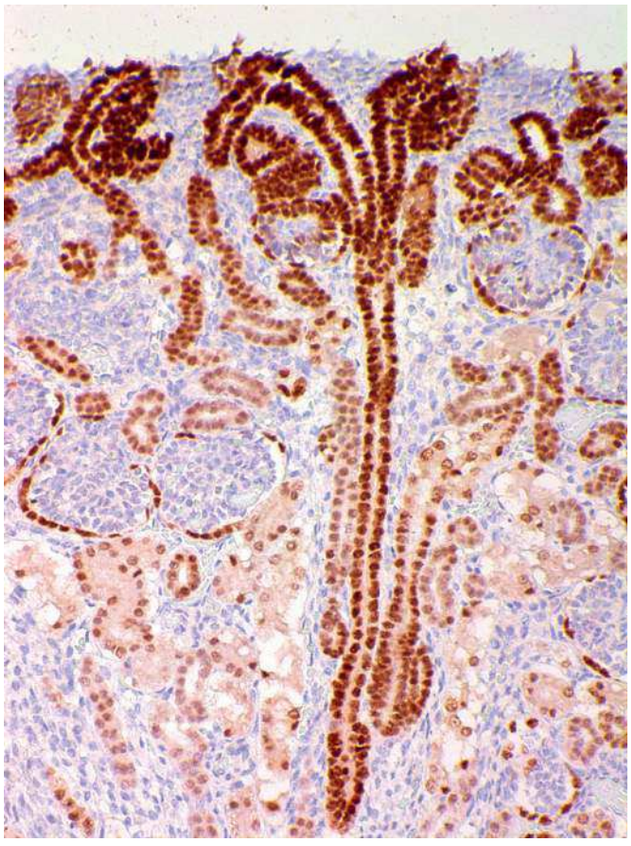

Figure 1.15 PAX2 immunohistochemistry: fetal kidney of 20 weeks' gestation showing prominent nuclear expression in ureteral epithelium and early nephron elements (including renal vesicles, comma- and S-bodies, and podocytes). Note the elongated ureteral growth. PAX2 is first downregulated in the differentiating podocytes and then the proximal and distal tubules. It becomes a persistent marker of parietal epithelial cells. (PAX IHC, × 200.)

membrane; one daughter cell retains the basal cytoplasmic process and retreats back into the epithelium; the other daughter cell remains in the lumen and eventually re-enters the epithelium a few cell diameters away. The function of this curious process is not clear.

Collecting Duct Differentiation

As mentioned, the continuous branching gives way to elongation of collecting ducts in later kidney development (see Figures 1.15 and 1.17). In the maturing medulla, the long straight collecting ducts run almost parallel. They appear patterned by "node retraction", in which the node (branch point) of a Y-shaped branch moves downwards, shortening the stalk of the Y, lengthening its arms and narrowing the divergence angle such that the Y becomes a V (Figure 1.19) (35). Considerable cellular remodeling occurs in the developing collecting system including the differentiation of aquaporin-2 (Aqp2)-positive principal cells and carbonic anhydrase-positive intercalated cells. The forkhead gene *Foxi1*, expressed in intercalated cells but not principal cells, appears to mediate the differentiation of intercalated cells from precursor cells (36). At least some precursor cells that give rise to

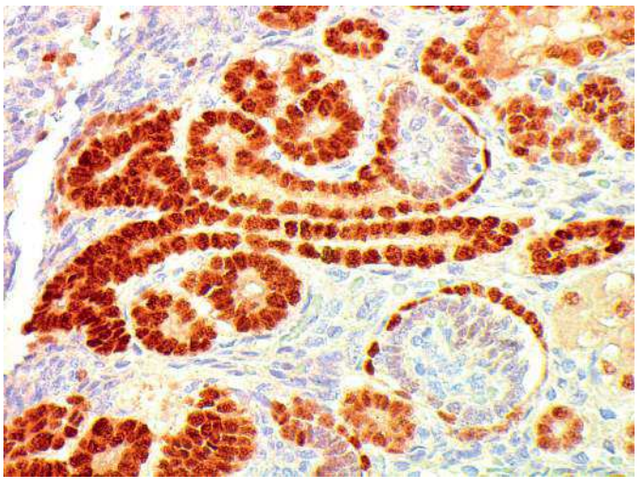

Figure 1.16 Nephrogenic zone in kidney of 20 weeks' gestation demonstrating PAX2 protein in nuclei of ureteral branches and ampullae, renal vesicles and parietal epithelial cells. (PAX IHC, × 400.)

intercalated cells have been reported to express Aqp2 (37). Notch signaling appears to be critical for principal cell differentiation as the absence of notch signaling confers an intercalated cell phenotype (38,39).

Metanephric Mesenchyme

Making a nephron requires several steps: specification of the metanephric mesenchyme, survival and proliferation of the nephron progenitors, differentiation of the progenitors to the renal vesicle, morphogenesis and patterning of early nephron segments, and terminal cell differentiation (40–42). These events occur within a "nephron niche" that broadly includes the ureteral ampulla, cap mesenchyme, pretubular aggreagate, renal vesicle, S-body, stroma, and endothelial progenitors. Most cells in the developing kidney derive from progenitor cells expressing *Osr1* (Figures 1.13 and 1.20). It is the earliest known marker of metanephric mesenchyme. Prior to UB invasion, *Osr1*-positive cells gives rise to both epithelial (*Pax2/Six2*+) and interstitial cell (*Foxd1*+) lineages. After UB induction, *Osr1*+ cells become confined to the cap mesenchyme nephron progenitor cells. A hierarchical cascade of transcription factors, including *Eya1, Six1, Pax2, Sall1*, and *Hox11* paralogs is essential for the metanephric mesenchyme mission of balancing self-renewal and differentiation of the nephron progenitor cells and the maintenance of *Gdnf* expression (Figure 1.20). NCAM1 (neural cell adhesion molecule) represents a potential valuable marker of renal progenitor cells (43,44). It is expressed in the cap mesenchyme and early nephron epithelial structures, such as the S-bodies, but not in mature nephron elements or ureteral branches (Figure 1.21A,B).

Cells expressing *Six2* and *Cited1* define a stem cell population capable of self-renewal and expansion within the cap mesenchyme. *Six2* expression keeps this nephron progenitor population in an undifferentiated state (45). The importance of this population is emphasized by studies showing that