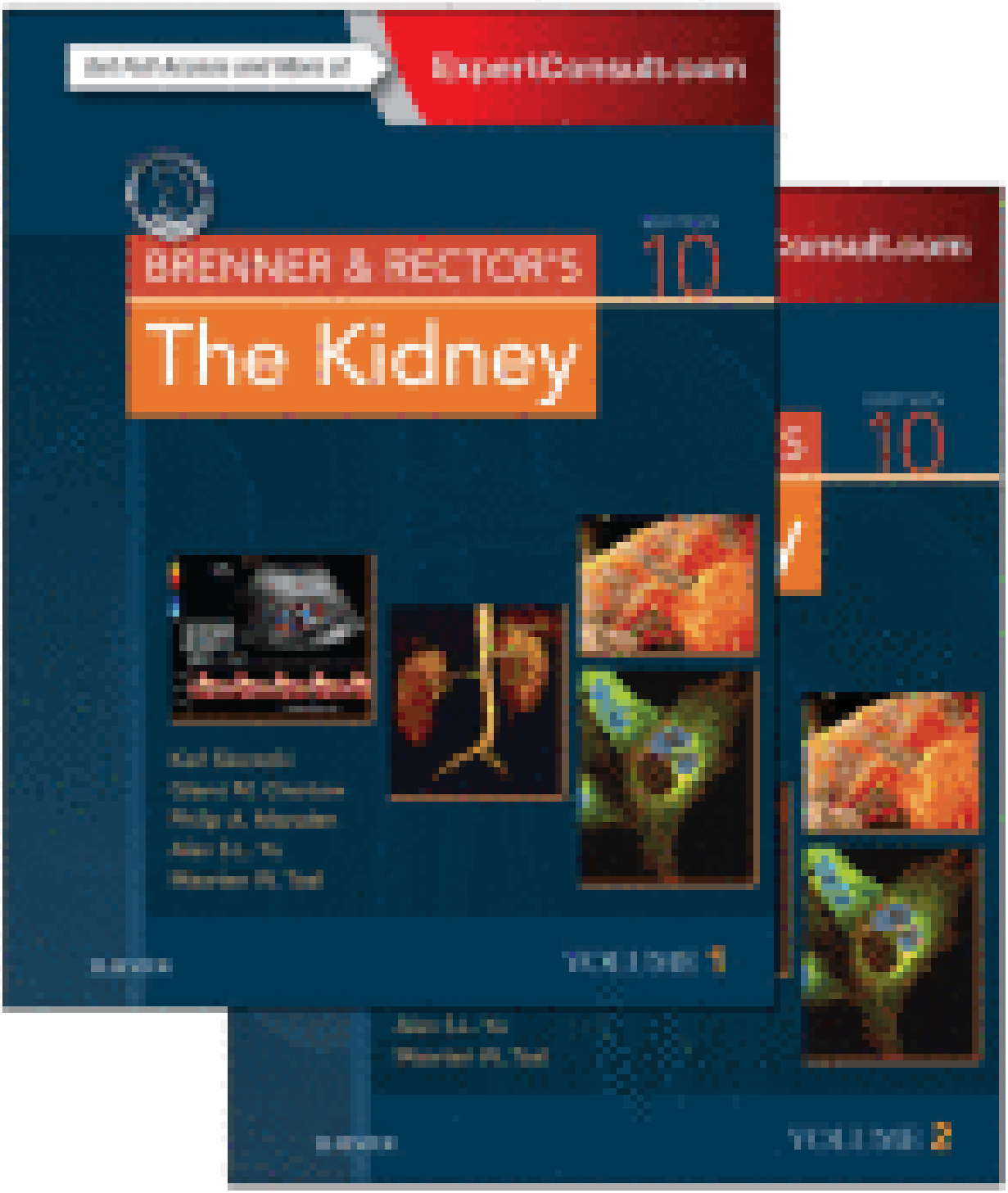
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The Kidney
2-Volume Set

Tenth Edition

BRENNER & RECTOR'S THE KIDNEY

VOLUME 1

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Foreword

Ten quadrennial editions and counting! This latest edition of Brenner and Rector's *The Kidney*, which comes 40 years after the first, is also the first in which I have had no formal role. The work of editing is now in the very capable hands of five exceptionally gifted and internationally dispersed former colleagues. It is perhaps fitting then to leave behind something of the history of how this textbook came into being. The year was 1972, the setting the Veterans Administration Medical Center at Fort Miley, perched on a high bluff overlooking the Golden Gate Bridge at the entrance to San Francisco Bay. I was then in my third year beyond renal physiology fellowship training, holding the position as Chief, Nephrology Section, overseeing a faculty of four and a single laboratory devoted to basic kidney research. Exploiting surface glomeruli in a unique strain of Wistar rats, using specially designed micropuncture techniques, our now classical studies of glomerular hemodynamics and permselectivity propelled me up the academic ladder such that a full professorship in the University of California system was soon earned. I was so self-confident and ambitious that new challenges and adventures were eagerly sought and considered.

But the one that presented itself on a Saturday morning in late 1972 could hardly have been imagined. After reviewing the week's laboratory data with my research team, I wandered, as I often did, into the nearby office of the Chair of Medicine, Marvin H. Sleisenger, whose warm and supportive words were always a treasured source of guidance and encouragement. On this particular morning's visit, I saw on his desk before him reams of long vertical galley proof of what was soon to become the first edition of a new textbook on gastroenterology, co-edited with John Fordtran. How wonderful it must feel, I remarked, to be in the position to oversee the organization and synthesis of a major field of internal medicine. He indeed expressed great pride and satisfaction in dealing with this challenge and, to my complete amazement, gazed up at me and suggested that this might be the appropriate time in my career to undertake a similar responsibility for a large-scale academic work in nephrology.

Flattered, of course, I left his office with little belief that I had the knowledge or capability to take on so formidable a challenge at this relatively early stage in my career. Not more than a week later, however, Albert Meier, Senior Editor at W.B. Saunders Publishing Company, was in my office urging me to set aside my reservations and undertake the responsibility for putting together a comprehensive compendium of nephrology, from basic science to clinical diagnosis and treatment of kidney disease. Weeks passed without decision into early 1973, when I learned that Floyd C. Rector, Jr., a world-renowned academic nephrologist, was moving to San Francisco to direct the Renal Division at the University of California, San Francisco. Imagine my excitement at the prospect of collaborating with this brilliant physician-scientist on a project of this magnitude and importance. Upon my sharing the notion with him, Dr. Rector was quick to agree that a two-volume textbook of nephrology based on fundamental physiologic principles was indeed needed, and we soon informed Saunders that a detailed outline of the scope and organization that reflected our combined personal insights and imagination would soon be forthcoming. All this was achieved in an informal 4-hour session in the living room of my Mill Valley home, where, over a lovely bottle of Napa Valley cabernet sauvignon and delicious, warm canapés prepared by my wife, Jane, we sketched out the five-section structure of a book that would remain unaltered over seven editions, namely, "Elements of Normal Renal Function," "Disturbances in Control of Body Fluid Volume and Composition," "Pathogenesis of Renal Disease," "Pathophysiology of Renal Disease," and "Management of the Patient with Renal Failure." Over the next few weeks, we added the filigree of specific chapter titles, prospective authors, timelines, and our shared editorial responsibilities and submitted the operational plan to Saunders for their executive consideration. Enthusiastic approval and contracts soon followed, and we were then busy with formal letters of invitation to authors (no e-mail in those days) for 49 chapters in nearly 2000 printed pages, with not a single turndown.

The first edition of *The Kidney* debuted at the ninth annual meeting of the American Society of Nephrology in November 1975, bearing the publication date of 1976. Acceptance was instantaneous and robust. Three subsequent editions with Dr. Rector appeared in 1980, 1984, and 1988, each extensively revised and expanded to reflect the remarkable progress in the field. I then served as sole editor for four editions, including an extensive structural redesign for the eighth edition, which consisted of 70 chapters in 12 sections. Among the newly crafted sections were the timely themes of "Epidemiology and Risk Factors in Kidney Disease," "Genetic Basis of Kidney Disease," and "Frontiers in Nephrology." The eighth edition also displayed cover art, tables, and figures redrawn in house in multicolor format and a fully functional electronic edition. In the preface to this eighth edition, which appeared in 2008, I wrote, "Just as blazing embers eventually grow dimmer, I recognize that now is the appropriate time to begin the orderly transition of responsibility for future editions…to a new generation of editors." An international team consisting of Glenn M. Chertow, Philip A. Marsden, Karl L. Skorecki, Maarten W. Taal, and Alan S. L. Yu joined me in crafting the ninth edition, to which two major new sections were added, "Pediatric Nephrology" and "Global Considerations in Kidney Disease." And for this tenth edition, which you are now

reading, these five editors have operated fully independently in producing this extensively updated and further expanded latest edition, featuring several novel new chapters, by far the best ever!

In addition to the refinements mentioned, what has come to be known as the "Brenner and Rector" project has grown into a very well received library of nephrology, consisting of discrete companion volumes designed to delve more deeply into specific areas of readership interest, including *Therapy in Nephrology and Hypertension*; *Chronic Kidney Disease, Dialysis, and Transplantation*; *Hypertension*; *Acute Renal Failure*; *Acid-Base and Electrolyte Disorders*; *Diagnostic Atlas of Renal Pathology*; *Molecular and Genetic Basis of Renal Disease*; and *Pocket Companion to Brenner and Rector's The Kidney*.

Nephrology has evolved dramatically over these past 40 years and will surely continue at an ever-quickening pace in the future. This will necessitate a full thrust into multimedia electronic formats such that updating new developments will appear more and more as a continuum. This will surely require new tools and editorial flexibility not yet tested. But therein may lie the project's greatest challenge.

Looking back, I could hardly have imagined the enormous success and respect this textbook project has enjoyed. Of course, full credit rests entirely with the authors of the chapters in each edition, whose enormous commitments of time and effort provided the outstanding scholarship and synthesis their respective areas demanded, along with invaluable comprehensive bibliographies, all of which served our devoted readership so well. My gratitude to them, our editorial staff, and the readers for their generous feedback over the years is unbounded. Playing a part in documenting the ever-more complex and expanding disciplines of renal science and medicine is among my life's greatest pleasures and challenges. If only I could again be a young student and have this magnificent new edition introduce me to the kidney's many wonders and enigmas.

Barry M. Brenner, MD

Preface

The tenth edition of *The Kidney* represents a turning point in the more than 40-year history of what has rightfully become a classic in nephrology. Barry Morton Brenner, co–founding editor with his distinguished colleague, Floyd Rector, and sole editor for the fourth through eighth editions, has shepherded an orderly transition of editorial stewardship to five of his fortunate trainees. We served as co-editors with Dr. Brenner on the ninth edition, for which Maarten W. Taal was a lead editor, and have now been fully entrusted with this precious legacy, buoyed by the mentorship and training that we have each received from Dr. Brenner.

The same sense of honor, mixed with trepidation, responsibility, and pride, that accompanied each of us as we entered the vaunted nephrology clinical and research program in Dr. Brenner's division at Brigham and Women's Hospital now accompanies us as we accept into our hands this "labor of love." Although this is the first edition for which Dr. Brenner is not an editor, his presence is palpable throughout the book. A fascinating history of *The Kidney* is described in the foreword by Dr. Brenner, and the narrative very much follows the exciting history of scientific discovery and clinical advances in the rather young clinical specialty of nephrology and our emerging knowledge of kidney biology. Dr. Brenner's imprint is also evident in so many of his own scientific discoveries and insights that have transformed our understanding of all aspects of the kidney in health and disease, as described by the authors throughout all the sections of the book. *The Kidney* continues to combine authoritative coverage of the most important topics of relevance to readers worldwide with the excitement of "a work in progress" presenting novel and transformative insights based on basic and clinical research and clinical paradigms that inform and improve medical care to patients with kidney disease in every corner of the world.

The more than 200 authors with whom we have had the great privilege of working have succeeded in transmitting not only a wealth of information, but also a sense of passion for the topics at hand. We hope that the reader will readily identify for each author the specific attraction that draws the author closer to the subject. These are myriad and diverse, ranging from the sheer and exquisite beauty of the architecture, structure, and substructure of the renal system, to the intricacies of cellular and molecular function, alongside advances in our understanding of disease pathogenesis at the most fundamental level, coupled with the opportunity to offer lifesaving clinical management with a global health perspective. Indeed, the authors reflect an international fellowship of dedicated researchers, scientists, and health professionals who find their expression in narrative text, images, illustrations, Web links, review questions, and references that constitute this tenth edition of *The Kidney*.

Most of all, the book is imbued with the inspiration of Dr. Brenner. We feel that it is this ingredient that guarantees the continued success of *The Kidney* in an era when other textbooks in all specialties are supplanted by a morass of other information sources. We, the editors and publishers, together with our authors, believe in the cardinal importance of a coherent and updated source of empowering information for students and devotees of the kidney, whether in the professional, teaching, or research domain.

To this end, the ninth edition of *The Kidney*, with Maarten W. Taal as lead editor, introduced several major changes that have proven enormously successful. Therefore we have retained and extended these innovations in the tenth edition. As befitting a living textbook, all chapters have been extensively updated or entirely rewritten. All of the authors are authorities in their respective fields, and many have accompanied *The Kidney* for several editions. However, new authors have been invited to provide refreshing perspectives on existing topics or to introduce brand-new areas relevant to kidney biology and health. One of the many examples is thorough consideration of our completely transformed understanding of sodium balance, resulting from the discovery of sodium stores whose very existence had been unknown and whose fluxes are under complex hormonal and growth factor regulation. By combining the classical and authoritative with transformative discovery and perspectives, *The Kidney* has positioned itself as the "go-to" reference and also the leading learning resource for kidney health and disease throughout the world. For example, a section on pediatric kidney disease was included in the ninth edition, and the positive feedback we received resulted in greater emphasis in the tenth edition. The extension of *The Kidney* into pediatric kidney disease will allow individuals and institutions throughout the world, sometimes with limited resources, to access information from a learning resource that covers kidney health and disease from pre-conception, through fetal and infant health, childhood, adulthood, and into old age. Similarly, the section on global perspectives has been expanded, and the chapter on ethical challenges has been deepened.

A number of practical considerations were also taken into account in the production of the tenth edition. Positive feedback and reviews have reinforced the overall organization into 14 sections and 87 chapters that take the reader from normal structure and function through to current and future challenges in the concluding section.

The authors have been asked to choose 50 key references for their respective chapters, whose citations will appear in the print edition. The online edition will in turn offer access to the full repertoire of references for each chapter, allowing scholarly primary assessment of each subject. As a new resource, we have included a set of board review–style

questions for those using *The Kidney* in preparation for certification and other examination purposes. As an educational resource, readers will be able to download figures for PowerPoint teaching purposes. We have also made an effort to adopt uniform terminology and nomenclature, in line with emerging consensus in the world kidney community. Thus, wherever possible, we have preferred terms such as *chronic kidney disease* and *acute kidney injury*, replacing the diverse and sometimes confusing terms that have peppered the literature in the past. Through Expert Consult, individuals who wish access to a physiology or disease topic at the most authoritative level will also be able to acquire separate chapters of interest, as might be the case for scientists and professionals outside of nephrology. Thus, through acquisition of *The Kidney*, individuals or institutions acquire a companion to accompany them on their journey in study, research, or patient care related to kidney health and disease.

Production of *The Kidney* is very much a team effort. The editors are indebted to the publication production team. Joan Ryan has served as our guide and lamppost beaconing the numerous contributors and providing expert input and support as Senior Content Development Specialist now for the ninth and tenth editions. Kate Dimock, Helene Caprari, and now Dolores Meloni have successfully assumed successive positions as Content Strategists, and Mary Pohlman as Senior Project Manager. These are but a few of the many members of the highly professional team at Elsevier, from whose wealth of experience the editors have benefited greatly.

None of this is possible without our authors, whose imprimatur, loyalty, and commitment to the highest standards continue to place *The Kidney* in its well-deserved position of international recognition. Through interactions with authors, we have also been able to strengthen long-standing bonds and to cultivate friendships. Most importantly, we owe a debt of gratitude to our readers, whose loyalty to and enthusiastic participation in each new edition energizes us as editors and reinforces our belief that the guiding spirit of Brenner and Rector for the subject matter and respect for the tradition initiated by the veritable "father" of *The Kidney*—Barry Morton Brenner—will continue to enliven this labor of love through many future editions.

On behalf of my co-editors, Maarten Taal, Glenn Chertow, Alan Yu, and Philip Marsden, I express tremendous gratification with the work that has become a major part of our lives and those of our families and friends and hope that the reader will also share this gratification upon partaking of *The Kidney*.

Karl Skorecki
Haifa, Israel

1 Embryology of the Kidney

Rizaldy P. Scott | Yoshiro Maezawa | Jordan Kreidberg | Susan E. Quaggin

CHAPTER OUTLINE

Over the past several decades, the identification of genes and molecular pathways required for normal kidney development has provided insight into our understanding of obvious developmental diseases such as renal agenesis and renal dysplasia. However, many of the genes identified have also been shown to play roles in adult-onset and acquired kidney diseases such as focal segmental glomerulosclerosis. The number of nephrons present in the kidney at birth, which is determined during fetal life, predicts the risk of kidney disease and hypertension later in life; a lower number is associated with greater risk.[1-3] Discovery of novel therapeutic targets and strategies to slow and reverse kidney diseases requires an understanding of the molecular mechanisms that underlie kidney development.

MAMMALIAN KIDNEY DEVELOPMENT: EMBRYOLOGY

DEVELOPMENT OF THE UROGENITAL SYSTEM

The vertebrate kidney derives from the intermediate mesoderm of the urogenital ridge, a structure found along the posterior wall of the abdomen in the developing fetus.[4] It develops in three successive stages known as the *pronephros,* the *mesonephros,* and the *metanephros* (Figure 1.1), although only the metanephros gives rise to the definitive adult kidney. However, earlier stages are required for development of other organs, such as the adrenal gland and gonad, that also develop within the urogenital ridge. Furthermore, many of the signaling pathways and genes that play important roles in the metanephric kidney appear to play parallel roles during earlier stages of renal development, in the pronephros and mesonephros. The pronephros consists of pronephric tubules and the pronephric duct (also known as the precursor to the wolffian duct) and develops from the rostralmost region of the urogenital ridge at 22 days of gestation (humans) and 8 days post coitum (dpc; mouse). It functions in the larval stages of amphibians and fish, but not in mammals. The mesonephros develops caudal to the pronephric tubules in the midsection of the urogenital ridge. The mesonephros becomes the functional excretory apparatus in lower vertebrates and may perform a filtering function during embryonic life in mammals. However, it largely degenerates before birth. Prior to its degeneration, endothelial, peritubular myoid, and steroidogenic cells

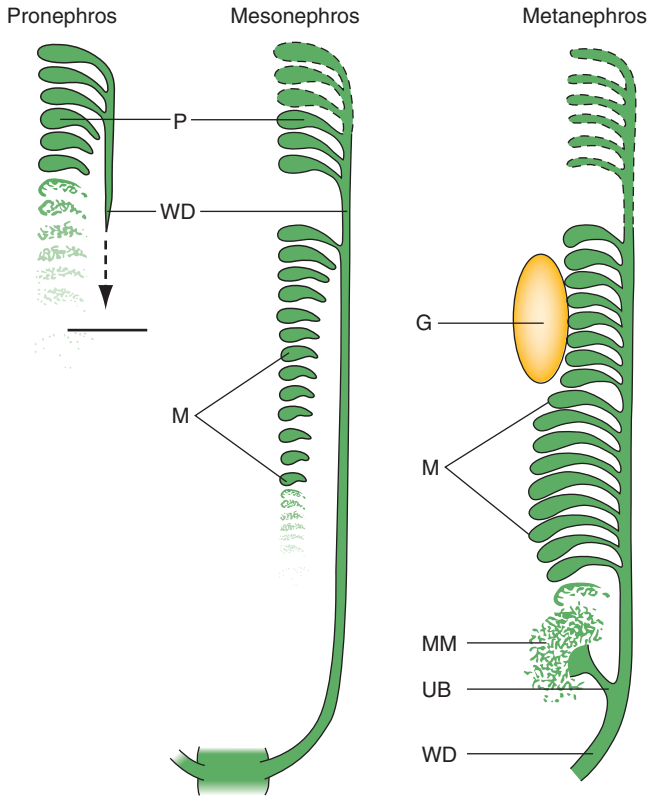

Figure 1.1 Three stages of mammalian kidney development. The pronephros (P) and mesonephros (M) develop in a rostral-to-caudal direction and the tubules are aligned adjacent to the wolffian or nephric duct (WD). The metanephros develops from an outgrowth of the distal end of the wolffian duct known as the ureteric bud epithelium (UB) and a cluster of cells known as the metanephric mesenchyme (MM). Cells migrate from the mesonephros (M) into the developing gonad (G), which develop in close association with each another. (Adapted from Saxen L: *Organogenesis of the kidney,* Cambridge, 1987, Cambridge University Press.)

from the mesonephros migrate into the adjacent adrenogonadal primordia, which ultimately form the adrenal gland and gonads.[5] Abnormal mesonephric migration leads to gonadal dysgenesis, a fact that underscores the intricate association between these organ systems during development and explains the common association of gonadal and renal defects in congenital syndromes.[6,7] In males, production of testosterone also induces the formation of seminal vesicles, tubules of the epididymis, and portions of the vas deferens from the wolffian duct.

DEVELOPMENT OF THE METANEPHROS

The metanephros, the third and final stage, gives rise to the definitive adult kidney of higher vertebrates; it results from a series of reciprocal inductive interactions that occur between the metanephric mesenchyme (MM) and the epithelial ureteric bud (UB) at the caudal end of the urogenital ridge. The UB is first visible as an outgrowth at the distal end of the wolffian duct at approximately 5 weeks of gestation in humans or 10.5 dpc in mice. The MM becomes histologically distinct from the surrounding mesenchyme and is found adjacent to the UB. Upon invasion of the MM by the UB, signals from the MM cause the UB to branch into a T-tubule (at around 11.5 dpc in mice) and then to undergo iterative dichotomous branching, giving rise to the urinary collecting duct system (Figure 1.2). Simultaneously, the UB sends reciprocal signals to the MM, which is induced to condense along the surface of the bud. Following condensation, a subset of MM cells aggregates adjacent and inferior to the tips of the branching UB. These collections of cells, known as *pretubular aggregates,* undergo mesenchymal-to-epithelial conversion to become the renal vesicle (Figure 1.3).

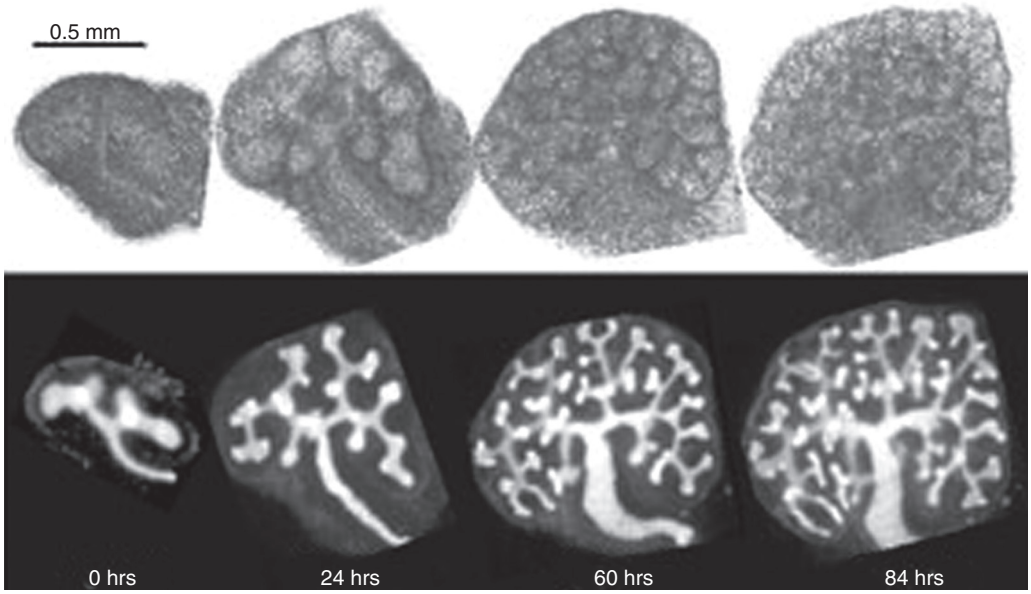

Figure 1.2 Organ culture of rat metanephroi dissected at T-tubule stage. Within 84 hours, dichotomous branching of the ureteric bud (UB) has occurred to provide the basic architecture of the kidney. *Bottom panel* is stained with *Dolichos biflorus* agglutinin—a lectin that binds specifically to UB cells. (Adapted from Saxen L: *Organogenesis of the kidney,* Cambridge, 1987, Cambridge University Press.)

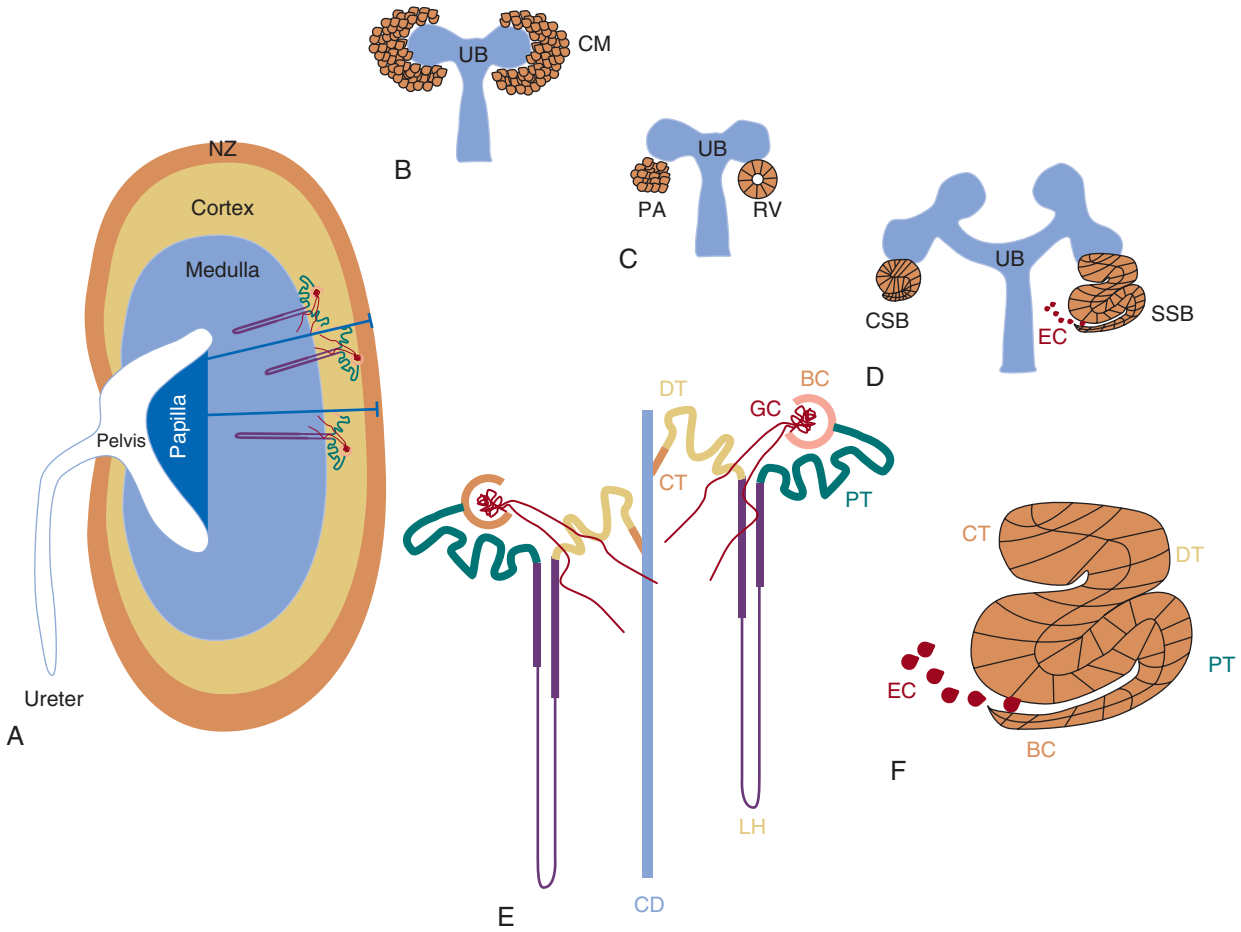

Figure 1.3 Overview of kidney development. A, Gross kidney histoarchitecture. NZ, nephrogenic zone. **B** through **E,** As described in the text, reciprocal interaction between the ureteric bud (UB) and metanephric mesenchyme results in a series of well-defined morphologic stages leading to formation of the nephron, including to the branching of the UB epithelium and the epithelialization of the metanephric mesenchyme into a highly patterned nephron. **F,** Distinctive segmentation of the S-shaped body defines the patterning of the nephron. BC, Bowman's capsule; CD, collecting duct; CM, cap mesenchyme; CSB, comma-shaped body; CT, connecting tubule; DT, distal tubule; EC, endothelial cells; LH, loop of Henle; PA, pretubular aggregate; PT, proximal tubule; SSB, S-shaped body.

DEVELOPMENT OF THE NEPHRON

The renal vesicle undergoes patterned segmentation and proceeds through a series of morphologic changes to form the glomerulus and components of the nephrogenic tubules from the proximal convoluted tubule, the loop of Henle, and the distal tubule. The renal vesicles undergo differentiation, passing through morphologically distinct stages starting from the comma-shaped body and proceeding to the S-shaped body, capillary loop, and mature stage, each step involving precise proximal-to-distal patterning and structural transformations (see Figure 1.3). Remarkably, this process is repeated 600,000 to 1 million times in each developing human kidney as new nephrons are sequentially born at the tips of the UB throughout fetal life.

The glomerulus develops from the most proximal end of the renal vesicle that is farthest from the UB tip.[8,9] Distinct cell types of the glomerulus can first be identified in the S-shaped body stage, in which presumptive podocytes appear as a columnar epithelial cell layer. A vascular cleft develops and separates the presumptive podocyte layer from more distal cells that will form the proximal tubule. Parietal epithelial cells differentiate and flatten to form Bowman's capsule, a structure that surrounds the urinary space and is continuous with the proximal tubular epithelium. Concurrently, endothelial cells migrate into the vascular cleft. Together with podocytes, the endothelial cells produce the glomerular basement membrane (GBM), a major component of the mature filtration barrier. Initially the podocytes are connected by intercellular tight junctions at their apical surfaces.[10] As glomerulogenesis proceeds, the podocytes revert to a mesenchymal-type phenotype, flatten, and spread out to cover the greater surface area of the growing glomerular capillary bed. They develop microtubule-based primary processes and actin-based secondary foot processes. During this time, the intercellular junctions become restricted to the basal aspect of each podocyte and eventually are replaced by a modified adherens junction–like structure known as the *slit diaphragm* (SD).[10] At the same time, foot processes from adjacent podocytes become highly interdigitated. The SDs are signaling hubs serving as the final layer of the glomerular filtration barrier.[11] Mesangial cell ingrowth follows the migration of endothelial cells and is required for development and patterning of the capillary loops that are found in normal glomeruli. The endothelial cells also flatten considerably, and capillary lumens are

formed owing to apoptosis of a subset of endothelial cells.[12] At the capillary loop stage, glomerular endothelial cells develop fenestrae, which are semipermeable transcellular pores common in capillary beds exposed to high hemodynamic flux. Positioning of the foot processes on the GBM and spreading of podocyte cell bodies are still incompletely understood but share many features of synapse formation and neuronal migration.[13-15]

In the mature stage, glomerulus, the podocytes, fenestrated endothelial cells, and intervening GBM compose the filtration barrier that separates the urinary from the blood space. Together, these components provide a size- and charge-selective barrier that permits free passage of small solutes and water but prevents the loss of larger molecules such as proteins. The mesangial cells are found between the capillary loops (approximately three per loop); they are required to provide ongoing structural support to the capillaries and possess smooth muscle cell–like characteristics that give them the capacity to contract, which may account for the dynamic properties of the glomerulus. The tubular portion of the nephron becomes segmented in a proximal-to-distal order, into the proximal convoluted tubule, the descending and ascending loops of Henle, and the distal convoluted tubule. The distal tubule is contiguous with the collecting duct, a derivative of the UB. Imaging and fate mapping analysis reveal that this interconnection results from the invasion of the UB by cells from the distal segments of nascent nephrons (around the S-shaped body stage).[16]

Although all segments of the nephron are present at birth and filtration occurs prior to birth, maturation of the tubule continues in the postnatal period. Increased expression levels of transporters, switch in transporter isoforms, alterations in paracellular transport mechanisms, and the development of permeability and biophysical properties of tubular membranes have all been observed to occur postnatally.[17] Although additional studies are needed, these observations emphasize the importance of considering developmental stage of the nephron in interpretation of renal transport and may explain the age of onset of symptoms in inherited transport disorders; some of these issues may be recapitulated in acute kidney injury.

THE NEPHROGENIC ZONE

After the first few rounds of branching of the UB and the concomitant induction of nephrons from the MM, the kidney subdivides into an outer cortical region, where nephrons are being induced, and an inner medullary region, where the collecting system will form. As growth continues, successive groups of nephrons are induced at the peripheral regions of the kidney known as the *nephrogenic zone* (Figure 1.4). Thus, within the developing kidney, the most mature nephrons are found in the innermost layers of the cortex, and the most immature nephrons in the most peripheral regions. At the extreme peripheral lining, under the renal capsule, a process that seems to recapitulate the induction of the original nephrons can be observed, whereby numerous UB-like structures are inducing areas of condensed mesenchyme. Indeed, whether there are significant molecular differences between the induction of the original nephrons and these subsequent inductive events is not known. A subpopulation of self-renewing mesenchymal cells immediately adjacent and inferior to the UB tips at the nephrogenic zone undergoes epithelial transformation, giving rise to new nephrons postnatally.[18,19]

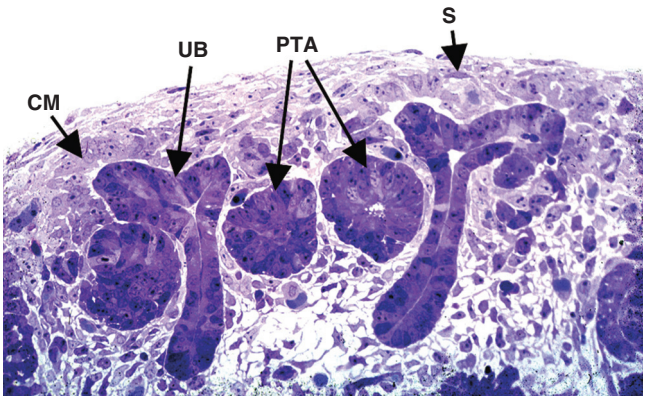

Figure 1.4 The nephrogenic zone. As described in the text, nephrons are continually produced in the nephrogenic zone throughout fetal life. CM, Condensing mesenchyme; PTA, pretubular aggregate; S, stromal cell lineage (spindle-shaped cells); UB, ureteric bud.

BRANCHING MORPHOGENESIS: DEVELOPMENT OF THE COLLECTING SYSTEM

The collecting system is composed of hundreds of tubules through which the filtrate produced by the nephrons is conducted out of the kidney and to the ureter and then the bladder. Water and salt resorption and excretion, ammonia transport, and H^+ secretion required for acid-base homeostasis also occur in the collecting ducts, under different regulatory mechanisms and using different transporters and channels from those that are active along tubular portions of the nephron. The collecting ducts are all derived from the original UB (Figure 1.5). Whereas each nephron is an individual unit separately induced and originating from a distinct pretubular aggregate, the collecting ducts are the product of branching morphogenesis from the UB. Considerable remodeling is involved in forming collecting ducts from branches of UB, and how this occurs remains incompletely understood.[20] The branching is highly patterned; the first several rounds are somewhat symmetric, additional rounds of branching are asymmetric, in which a main trunk of the collecting duct continues to extend toward the nephrogenic zone but smaller buds branch as they induce new nephrons within the nephrogenic zone. Originally, the UB derivatives are branching within a surrounding mesenchyme. Ultimately, they form a funnel-shaped structure in which cone-shaped groupings of ducts or papillae sit within a funnel or calyx that drains into the ureter. The mouse kidney has a single papilla and calyx, but a human kidney has 8 to 10 papillae, each of which drains into a minor calyx, with several minor calyces draining into a smaller number of major calyces.

RENAL STROMA AND INTERSTITIAL POPULATIONS

For decades in classic embryologic studies of kidney development, emphasis was placed on the reciprocal inductive

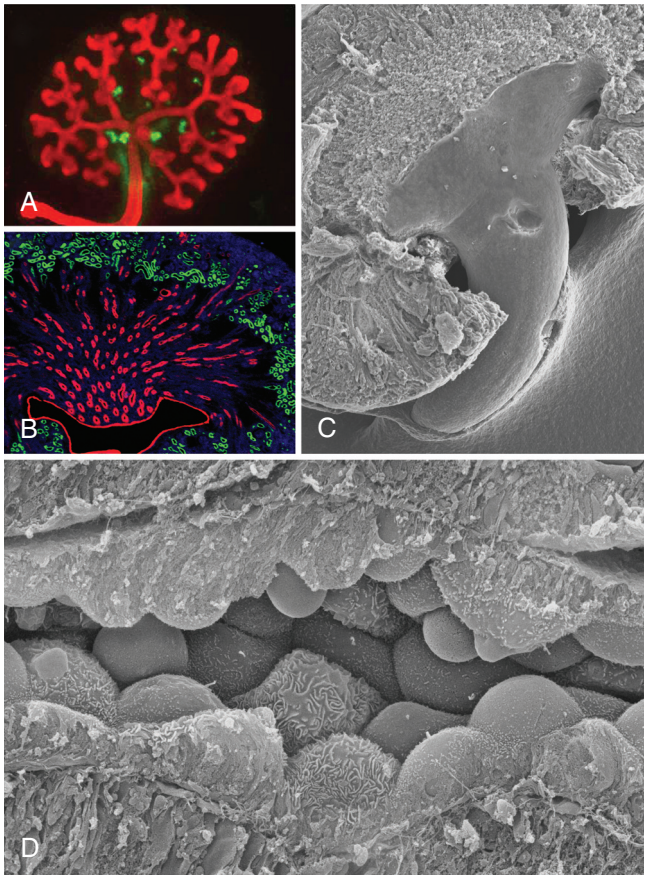

Figure 1.5 Collecting duct system. The branching ureteric epithelial lineage gives rise to the collecting duct system. **A,** E12.5 mouse embryonic kidney explant grown in vitro for 2 days and **B,** neonatal mouse kidney section, stained for the ureteric epithelium and collecting ducts (pan-cytokeratin, *red*) and the nascent proximal tubules (Lotus lectin, *green*). **C,** Scanning electron micrograph of a hemisected adult mouse kidney showing the funnel-shaped renal papillae. **D,** Scanning electron micrograph of a collecting duct showing smooth principal cells and reticulated intercalated cells.

signals between MM and UB. However, in later years, interest has arisen in the stromal cell as a key regulator of nephrogenesis.[9,21-23] Stromal cells also derive from the MM but are not induced to condense by the UB. Two distinct populations of stromal cells have been described: Cortical stromal cells exist as a thin layer beneath the renal capsule and medullary stromal cells populate the interstitial space between the collecting ducts and tubules (Figure 1.6). Cortical stromal cells also surround the condensates and provide signals required for UB branching and patterning of the developing kidney. Disruption or loss of these stromal cells leads to failure of UB branching, a reduction in nephron number, and disrupted patterning of nephric units with failure of cortical-medullary boundary formation. A reciprocal signaling loop from the UB exists to properly pattern stromal cell populations. Loss of these UB-derived signals leads to a buildup of stromal cells beneath the capsule that is several layers thick. As nephrogenesis proceeds, stromal cells differentiate into peritubular interstitial cells and pericytes that are required for vascular remodeling and for production of extracellular matrix responsible for proper nephric formation.[23] These cells migrate from their positions around the condensates to areas between the developing nephrons within the medulla. Although stromal cells are derived from the MM cells, it remains unclear whether stromal cells and nephric lineages arise from a common progenitor MM cell.

DEVELOPMENT OF THE VASCULATURE

The microcirculations of the kidney include the specialized glomerular capillary system responsible for production of the ultrafiltrate and the *vasa recta,* peritubular capillaries involved in the countercurrent mechanism. In the adult, each kidney receives 10% of the cardiac output. Vasculogenesis and angiogenesis have been described as two distinct processes in blood vessel formation. *Vasculogenesis* refers to de novo differentiation of previously nonvascular cells into structures that resemble capillary beds, whereas *angiogenesis* refers to sprouting from these early beds to form mature vessel structures including arteries, veins, and capillaries. Both processes are involved in development of the renal vasculature. At the time of UB invasion at 11 dpc (all timing given is for mice), the MM is avascular, but by 12 dpc a rich capillary network is present, and by 14 dpc vascularized glomeruli are present.

Transplantation experiments support a model whereby endothelial progenitors within the MM give rise to renal vessels in situ,[24] although the origin of large blood vessels is still debated. At 13 dpc capillaries form networks around the developing nephric tubules, and by 14 dpc the hilar artery and first-order interlobar renal artery branches can be identified. These branches will form the corticomedullary arcades and the interlobular arteries that branch from them. Further branching produces the glomerular afferent arterioles. From 13.5 dpc onward, endothelial cells migrate into the vascular cleft of developing glomeruli, where they undergo differentiation to form the glomerular capillary loops (Figure 1.7). The efferent arterioles carry blood away from the glomerulus to a system of fenestrated peritubular capillaries that are in close contact with the adjacent tubules and receive filtered water and solutes reabsorbed from the filtrate.[25] These capillaries have few pericytes. In comparison, the vasa recta, which surround the medullary tubules and are involved in urinary concentration, are also fenestrated but have more pericytes. They arise from the efferent arterioles of deep glomeruli.[26] The peritubular capillary system surrounding the proximal tubules is well developed in the late fetal period, whereas the vasa recta mature 1 to 3 weeks postnatally.

MODEL SYSTEMS TO STUDY KIDNEY DEVELOPMENT

ORGAN CULTURE

THE KIDNEY ORGAN CULTURE SYSTEM: CLASSIC STUDIES

Metanephric kidney organ culture (Figures 1.8 and 1.9) formed the basis for extensive classic studies of embryonic induction. Parameters of induction such as the temporal and physical constraints on exposure of the inductive tissue

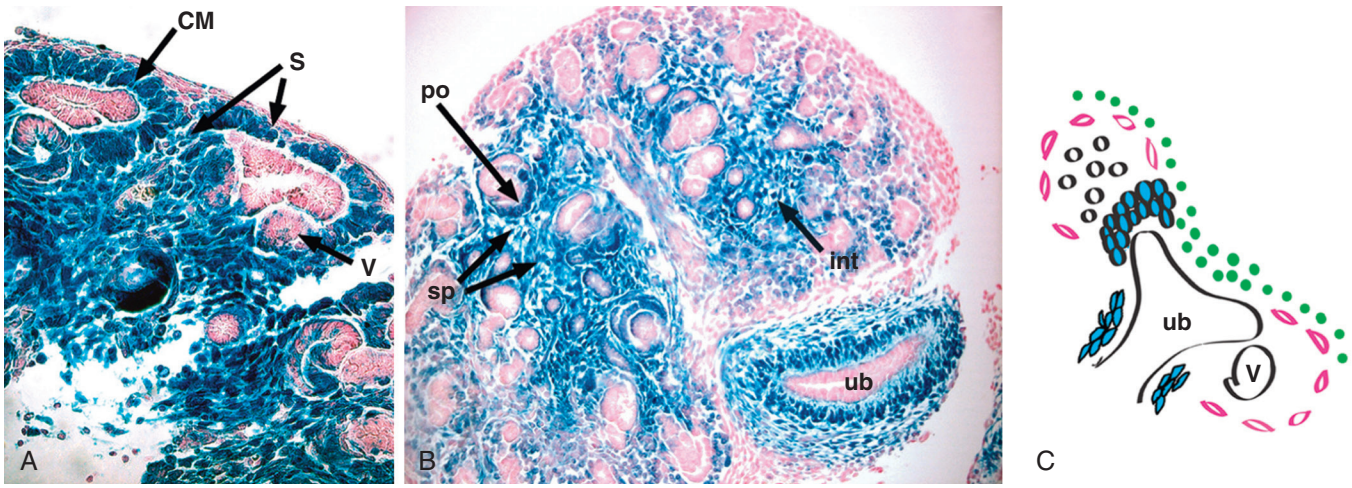

Figure 1.6 Populations of cells within the metanephric mesenchyme. As described in the text, these populations are defined by morphologic and molecular characteristics. Metanephroi from a 14.5 dpc Tcf21-LacZ mouse **(A)** and a 15.5 dpc Tcf21-LacZ mouse **(B)** are stained for β-galactosidase activity. Tcf21-expressing cells stain *blue*. Stromal cells (S; *pink* in **C**) are seen surrounding condensing mesenchyme (CM). Nephrogenic population (*green* in **C**) remains unstained. By 15.5 dpc a well-developed interstitial compartment is seen and consists of peritubular fibroblasts, medullary fibroblasts, and pericytes. Loose and condensed mesenchymal cells are also observed around the stalk of the ureteric bud in **B**. v, Renal vesicle; po, podocyte precursors; sp, stromal pericytes; int, interstitium. **C,** Schematic diagram of mesenchymal populations includes the nephrogenic precursors (in *green*), uninduced mesenchyme (*white*), condensing mesenchyme around the UB tips and stalk (*blue*), and stromal cell lineage (*pink*). (Reproduced with permission from *Developmental Dynamics*.)

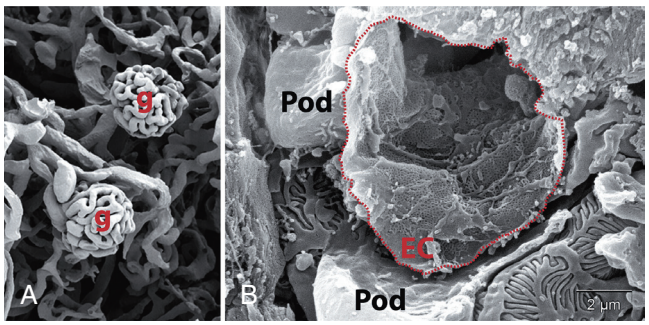

Figure 1.7 Renal vasculature. A, Corrosion resin cast of renal vasculature revealing the highly convoluted assembly of the glomerular capillaries (g). **B,** Scanning electron micrograph of a glomerulus with an exposed endothelial lumen (*dashed outlined*) showing fenestrations. EC, Endothelial cell; Pod, podocyte. (Corrosion cast electron micrograph courtesy of Fred Hossler, Department of Anatomy and Cell Biology, East Tennessee State University.)

to the mesenchyme were determined, as were the times during which various tubular elements of the nephron were first observed in culture.

MUTANT PHENOTYPIC ANALYSES

As originally shown by Grobstein, Saxen, and their colleagues in classic studies of embryonic induction, the two major components of the metanephric kidney, the MM and the UB, could be separated from each other, and the isolated mesenchyme could be induced to form nephron-like tubules by a selected set of other embryonic tissues, the best example of which is embryonic neural tube.[4,27] When neural tube is used to induce the separated mesenchyme, there is terminal differentiation of the mesenchyme into tubules, but not significant tissue expansion. In contrast, intact metanephric rudiments can grow more extensively, displaying both sustained UB branching and early induction of nephrons even when cultured for a week. The isolated mesenchyme experiment has proved useful in the analysis of renal agenesis phenotypes, in which there is no outgrowth of the UB. In these cases, the mesenchyme can be placed in contact with neural tube to determine whether it has the intrinsic ability to differentiate. Most often, when renal agenesis is due to the mutation of a transcription factor gene, tubular induction is not rescued by neural tube, as could be predicted for transcription factors which would be expected to act in a cell-autonomous fashion.[28] In the converse situation, in which renal agenesis is caused by loss of a gene function in the UB (e.g., *Emx2* in the mouse), it is usually possible for embryonic neural tube to induce tubule formation in isolated mesenchymes.[29] Therefore, the organ culture induction assay can be used to test hypotheses concerning whether a particular gene is required in the UB or the MM. As chemical inhibitors specific for various signal transduction pathways have been synthesized and become available, it has been possible to add them to organ cultures and observe effects that are informative about the roles of specific pathways in development of the kidney. Examples are the uses of drugs to block the Erk/MAP kinase, PI3K/Akt, and Notch signaling pathways in renal explant cultures.[30-32]

ANTISENSE OLIGONUCLEOTIDES AND siRNA IN ORGAN CULTURE

Several studies have described the use of antisense oligonucleotides and of siRNA (small interfering, or silencing, RNA) molecules to inhibit gene expression in kidney organ cultures. Among the earliest of these was the inhibition of the low-affinity nerve growth factor receptor, p75 or NGFR, by antisense oligonucleotides,[33] a treatment that decreased the growth of cultured embryonic kidneys. A subsequent study could not duplicate this phenotype,[34] although there were possible differences in experimental techniques.[35] An

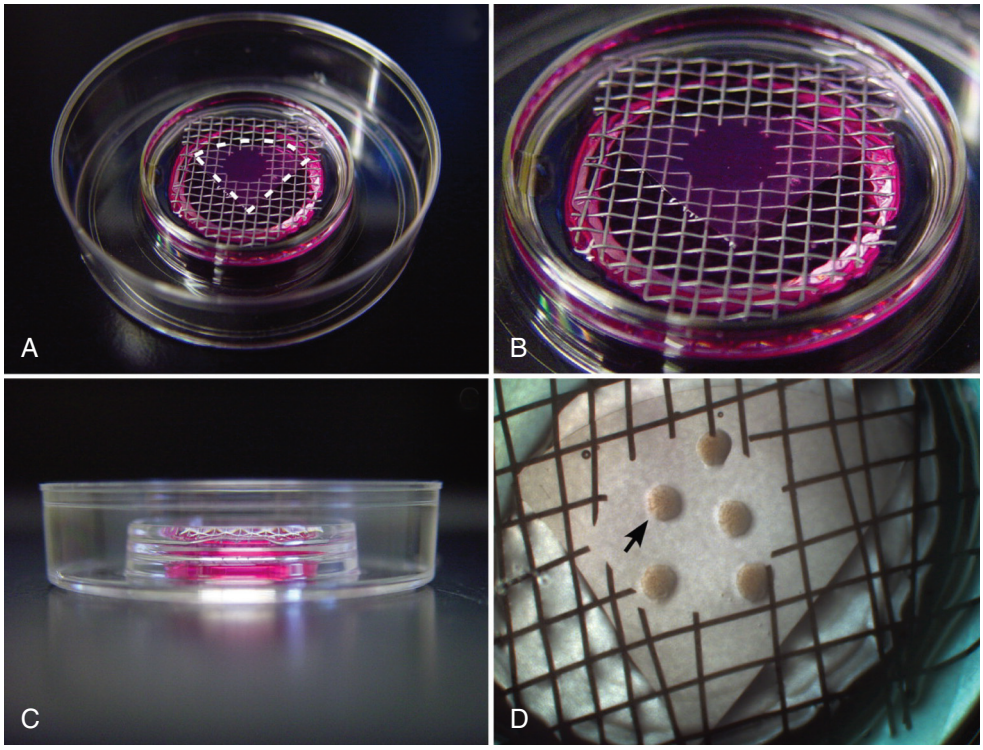

Figure 1.8 Metanephric organ explants Top **(A, B)** and lateral **(C)** views of a kidney organ culture in a center-well dish. Embryonic kidney explants are grown at the air-growth medium interface on top of a floating porous polycarbonate filter (*dashed lines* in **A**) supported on a metal mesh. **D,** Kidneys grown after 4 days of culture. (Reproduced with permission from Barak H, Boyle SC: Organ culture and immunostaining of mouse embryonic kidneys. *Cold Spring Harb Protoc* 2011[1]:pdb.prot5558, 2011.)

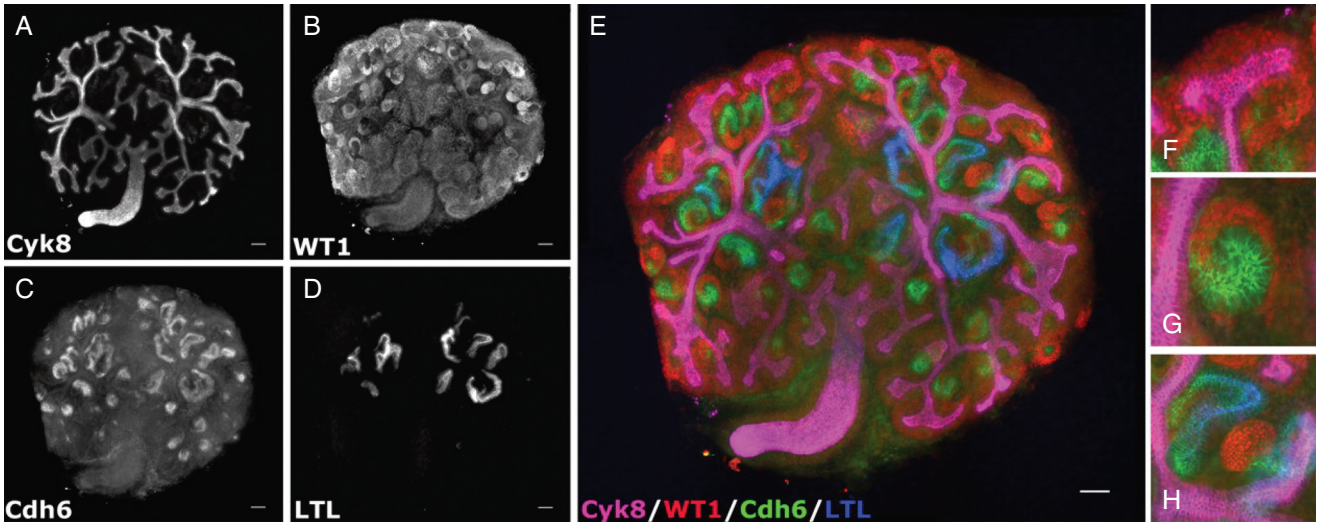

Figure 1.9 Recapitulation of branching and nephrogenesis in renal explant cultures. A, Ureteric tree stained for cytokeratin 8 (Cyk8). **B,** Condensed metanephric mesenchyme stained for WT1. **C,** Epithelial derivatives of the metanephric mesenchyme stained for E-cadherin (Cdh6). **D,** Proximal tubules stained with *Lotus tetraglobulus* lectin (LTL). **E,** Merged image of **A** through **D. F,** WT1-expressing cells represent the nephron progenitor cells that surround the ureteric bud. **G,** Cdh6-expression marks the mesenchyme-to-epithelial transformation of nephron progenitor cells. **H,** Early patterning of nascent nephrons along a proximodistal axis. **E-H,** Cyk8 (*magenta*), WT1 (*red*), Cdh6 (*green*) and LTL (*blue*). (Reproduced with permission from Barak H, Boyle SC: Organ culture and immunostaining of mouse embryonic kidneys. *Cold Spring Harb Protoc* 2011[1]:pdb.prot5558, 2011.)

additional study using antisense oligonucleotides to *Pax2* also showed this gene to be crucial in epithelialization of the MM.[36,37] Antisense morpholinos modified with an octaguanidine dendrimer moiety to facilitate cell uptake have been used to target Wilms' tumor-1 gene (*Wt1*) in kidney explant cultures. This morpholino-based knockdown strategy allowed the identification of WT1 transcriptional targets in nephron progenitors, which was technically impossible in conventional *Wt1* knockout mice because of renal agenesis.[38] Co-transported with synthetic delivery

peptides, antisense morpholinos have also been used to investigate the negative regulation of ureteric branching morphogenesis by semaphorin3a (Sema3a).[39,40] Gene knockdown using siRNA has also been used to demonstrate the importance of *Wt1* and *Pax2* in nephrogenesis in whole organ and dissociated embryonic kidneys.[38,41,42] Similar siRNA-based knockdown strategies have been successfully used to demonstrate the importance of fibronectin, Dact2, and estrogen-related receptor γ (Esrrg) in ureteric branching in whole embryonic renal explant cultures.[43-45]

ORGAN CULTURE MICROINJECTION

Microinjection in kidney explant cultures can be used to selectively target gene expression using a variety of reagents (plasmid constructs, viruses, and siRNA) in either the MM or the branching ureteric epithelia, depending on the site of injection.[46,47] Retroviruses encoding mutants of polycystin-1 were used to demonstrate that polycystin-1 is required for normal ureteric branching patterns.[48] Microinjection followed by electroporation of DNA plasmid constructs has been used to overexpress *GDNF* (glial cell–derived neurotrophic factor), *Wt1, Pax2, Vegfa,* and *Robo2* in the MM and to assess the role of these genes in ureteric branching and early nephron induction.[47,49]

TRANSGENIC AND KNOCKOUT MOUSE MODELS

Over the past two decades, the generation and analysis of knockout and transgenic mice have provided tremendous insight into kidney development (Table 1.1).[50,51] Although homologous recombination to delete genes within the germline, also known as standard "knockout" technology, has provided information about the biologic functions of many genes in kidney development, it has several disadvantages. Disruption of gene function in embryonic stem (ES) cells may result in embryonic or perinatal death, precluding the functional analysis of the gene in the kidney that develops relatively late in fetal life. Additionally, many genes are expressed in multiple cell types, and the resulting knockout phenotypes can be complex and difficult or impossible to dissect. The ability to limit gene targeting to specific renal cell types overcomes some of these problems, and the temporal control of gene expression permits more precise characterization of a gene's function. A number of mouse lines may be used to target specific kidney cell lineages (Table 1.2; Figure 1.10). As with any experimental procedure, numerous caveats must be taken into account in the interpretation of data.[52,53] These include determining the completeness of excision at the locus of interest, the timing and extrarenal expression of the promoters, and general toxicity of expressed proteins to the cell of interest. In spite of these issues, tissue-specific conditional gene targeting strategies remain powerful tools to study gene functions. The next generation of targeting includes improved efficiency using bacterial artificial chromosome (BAC) targeting approaches, siRNA and microRNA (miRNA) approaches, and large genomewide targeting efforts already under way at many academic and pharmaceutical institutions.

In contrast to gene targeting experiments in which the gene is known at the beginning of the experiment (*reverse genetics*), random mutagenesis represents a complimentary phenotype-driven approach (*forward genetics*) to study the physiologic relevance of certain genes. Random mutations are introduced into the genome at high efficiency by chemical or gene trap mutagenesis. Consecutively, large numbers of animals are screened systematically for specific phenotypes of interest. As soon as a phenotype is identified, test breeding is used to confirm the genetic nature of the trait. Chromosomal mapping and positional cloning are then used to determine the identity of the culprit mutant gene. There are two major advantages of genomewide approaches over reverse genetics. First, most knockouts lead to major gene disruptions, which may not be relevant to the subtle gene alterations that underlie human renal disease. Second, many of the complex traits underlying congenital anomalies and acquired diseases of the kidney are unknown, making predictions about the nature of the genes that are involved in these diseases difficult.

One of the most powerful and well-characterized mutagens in the mouse is the chemical mutagen *N*-ethyl-*N*-nitrosourea (ENU). It acts through random alkylation of nucleic acids, inducing point mutations in spermatogonial stem cells of injected male mice.[54,55] ENU mutagenesis introduces multiple point mutations within the spermatogonia of the male, which is then bred to a female mouse of different genetic background. Resultant offspring are screened for renal phenotypes of interest (e.g., dysplastic, cystic) and

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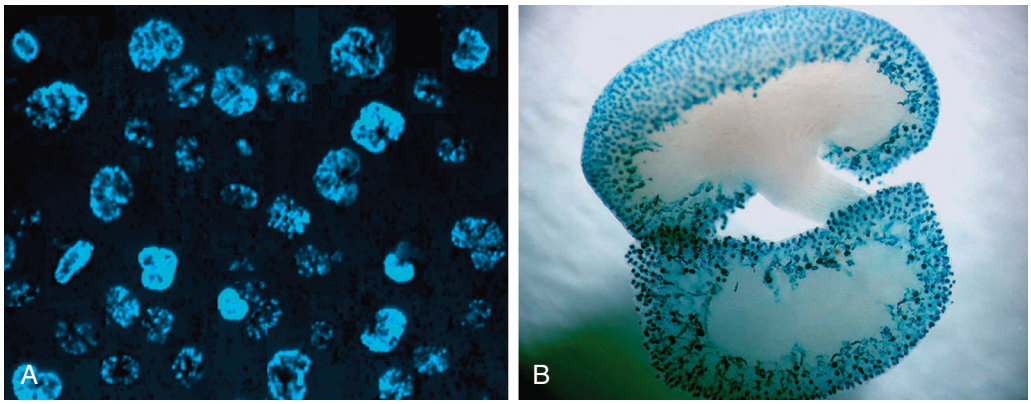

Figure 1.10 Glomeruli expressing **(A)** cyan fluorescent protein (CFP) or **(B)** β-galactosidase. Transgenic mice were generated using the nephrin promoter to direct expression of either CFP or β-galactosidase specifically in developing and mature podocytes.

Table 1.1 Summary of Knockout and Transgenic Models for Kidney Development

Gene Mutation or Knockout	Renal and Urogenital Tract Phenotypes, Other Tissues Affected	Associated Human Disease(s)	Reference(s)
Renal Aplasia (Variable)			
CTNNB1 (β-catenin)	Renal agenesis or severe renal hypoplasia, premature differentiation of UB epithelia (UB selective)	Colorectal cancer, hepatoblastoma, hepatocellular cancer	142
Emx2	Complete absence of urogenital system		29
Emx2, PAX2	Duplicated kidneys and ureter, ureteral obstruction	CAKUT, VUR	401
Etv4, Etv5	Renal agenesis or severe renal hypodysplasia		76,166
EYA1 (Eyes absent-1)	Renal agenesis, lack of UB branching and mesenchymal condensation	Branchiootorenal syndrome (brachial fistulas, deafness)	96, 110
Fgf9, Fgf20	Renal agenesis		230
Fgf10, GDNF, Gfra1	Renal agenesis		172
Fgfr1, Fgfr2	Renal agenesis (MM selective)		240
FRAS1, FREM1, FREM2	UB failure, defect of GDNF expression	Fraser's syndrome (cryptophthalmos, syndactyly, CAKUT); Manitoba-oculotrichoanal (MOTA) syndrome	122, 123, 178-180
GATA3	Renal agenesis, gonad dysgenesis (null mutation); ectopic ureteric budding, kidney, hydroureter (UB selective)	Hypoparathyroidism, sensorineural deafness, and renal dysplasia (HDRS) syndrome; autoimmune disease (rheumatoid arthritis)	136, 137, 139, 140
Gdf11 (growth differentiation factor 11)	UB failure, skeletal defects		102, 402
GDNF, Gfra1, RET	Renal agenesis or rudimentary kidneys, aganglionic megacolon	Hirschsprung disease, multiple endocrine neoplasm type IIA/B (MEN2A/MEN2B), and familial medullary thyroid carcinoma (FMTC)	103-106, 118, 119, 403-406,
GLI3	Renal agenesis, severe renal agenesis, absence of renal medulla and papilla	Pallister-Hall (PH) syndrome (polydactyly, imperforate anus, abnormal kidneys, defects in the gastrointestinal tract, larynx, and epiglottis)	212, 213
Grem1 (Gremlin)	Renal agenesis; apoptosis of the MM		107
GRIP1	Renal agenesis	Fraser's syndrome	182-184
Hox-A11/D11	Distal limbs, vas deferens		407
Hs2st1 (heparan sulfate 2 O-sulfotransferase 1)	Lack of UB branching and mesenchymal condensation		408
Isl1 (islet1)	Renal agenesis, renal hypoplasia, hydroureter (MM selective)		409
ITGA8 (integrin α_8)	Renal agenesis, renal hypodysplasia	Fraser's syndrome	124
Itgb1 (integrin β_1)	Disrupted UB branching, bilateral renal agenesis, hypoplastic collecting duct system (collecting duct selective); podocyte dedifferentiation (podocyte selective)	Fraser's syndrome	134, 410, 411
Kif26b	Renal agenesis, failed UB attraction to the MM		125
Lamc1	UB failure, delayed nephrogenesis, water transport defects		185
LHX1/LIM1	Renal agenesis (null mutant); renal hypoplasia, UB branching defect, hydronephrosis, distal ureter obstruction (UB selective); arrested nephrogenesis, nephron patterning defects (MM selective)	Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome (müllerian duct agenesis)	97, 412, 413
LRP4	Delayed UB induction, failed MM induction, syndactyly, oligodactyly	Cenani-Lenz syndrome	414-417

Table 1.1 Summary of Knockout and Transgenic Models for Kidney Development (Continued)

Gene Mutation or Knockout	Renal and Urogenital Tract Phenotypes, Other Tissues Affected	Associated Human Disease(s)	Reference(s)
Npnt (nephronectin)	Delay of UB invasion into MM		126
Osr1/Odd1	Lack of MM, adrenal gland, gonads, defects in formation of pericardium and atrial septum		98, 109
PAX2	Renal hypoplasia, VUR	CAKUT, VUR, optic nerve colobomas	36, 37
PAX2, PAX8	Defect in intermediate mesoderm transition, failure of pronephric duct formation	CAKUT, VUR, optic nerve colobomas	418
PTF1A (pancreas transcription factor 1α subunit/Danforth short-tail)	Failure of UB induction; anal atresia, persistent cloaca, skeletal malformation	Pancreatic and cerebellar agenesis; diabetes mellitus	419-421
Retinoic acid receptors (*Rara, Rarb2*)	Renal hypoplasia, dysplasia, hydronephrosis, skeletal and multiple visceral abnormalities		7, 9, 68
SALL1	Renal agenesis, severe renal hypodysplasia	Townes-Brock syndrome (anal, renal, limb, ear anomalies)	99, 422
SHH (Sonic hedgehog)	Bilateral or unilateral renal agenesis, unilateral ectopic dysplastic kidney, defective ureteral stromal differentiation	Vertebral defects, anal atresia, cardiac defects, tracheoesophageal fistula, renal anomalies, and limb abnormalities (VACTERL) syndrome	209
SIX1	Lack of UB branching and mesenchymal condensation	Branchiootorenal syndrome	96, 110
SOX8, SOX9	Renal genesis, renal hypoplasia	Camptomelic dysplasia (limb and skeletal defects, abnormal gonad development)	423
WT1	Renal and gonadal agenesis, severe lung, heart, spleen, adrenal, and mesothelial abnormalities	Wilms' tumor, aniridia, genitourinary abnormalities, and retardation (WAGR) syndrome; Denys-Drash syndrome	28, 38, 337, 338
Hypoplasia/Dysplasia/Low Nephron Mass			
Adamts1	Hypoplasia of the renal medulla, hydronephrosis		250, 253
Adamts1, Adamts4	Hypoplasia of the renal medulla, hydronephrosis		254
Agtr2 (angiotensin II type-2 receptor)	Various collecting system defects	CAKUT	202, 321, 322
Ald1a2/Raldh2 (retinal dehydrogenase)	Renal hypoplasia, hydronephrosis, ectopic ureter		139
BMP1RA/Alk3	Hypoplasia of renal medulla, fewer UB branches (UB selective)	Juvenile polyposis syndrome	200
Bmp7	Reduced MM survival		225
Cdc42, Yap	Renal hypoplasia, oligonephronia, defects in mesenchyme to epithelial transition (CM selective)		424
Cfl1, Dstn (cofilin1, destrin)	Renal hypodysplasia, ureter duplication		425
CTNNB1 (β-catenin)	Severely hypoplastic kidney, lack of nephrogenic zone and S-shaped body (CM selective)	Colorectal cancer, hepatoblastoma	223
DICER1	Renal hypoplasia, dysplasia, cysts (UB selective); renal hypoplasia characterized by premature termination of nephrogenesis (MM selective)	Pleopulmonary blastoma	426
Dkk1 (Dickkopf 1)	Overgrown renal papilla (renal tubule and collecting duct restricted)		196
Dlg1, Cask	Renal hypoplasia and dysplasia, premature depletion of nephrogenic precursor cells		326, 427

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Table 1.1 Summary of Knockout and Transgenic Models for Kidney Development (Continued)

Gene Mutation or Knockout	Renal and Urogenital Tract Phenotypes, Other Tissues Affected	Associated Human Disease(s)	Reference(s)
Egfr (epidermal growth factor receptor)	Hypoplasia of the renal papilla, moderate polyuria, and urine concentration defects		194
Esrrg	Agenesis of renal papilla		43
Fat4	Failed nephrogenesis (mesenchyme-to-epithelial transition), expansion of nephrogenic precursor zone (stroma selective)		274
Fgf7	Small kidneys, reduction in nephron number		198
FGF8	Renal dysplasia, arrested nephrogenesis at pretubular aggregate stage (MM selective)	Kallmann's syndrome, hypogonadism	428, 429
Fgf10	Renal hypoplasia, multiorgan developmental defects including the lungs, limb, thyroid, pituitary, and salivary glands		199
Fgfr1, Fgfr2	Renal agenesis (MM selective)		240
Fgfr2	Renal hypoplasia, hydronephrosis (UB selective)		69
FOXC2	Renal hypoplasia	AD lymphedema-distichiasis syndrome	214, 430
Foxd1 (BF-2)	Accumulation of undifferentiated CM, attenuated UB branching, stromal patterning defects		22, 266, 268
Foxd1	Mild renal hypoplasia (UB selective)		431
Fzd4, Fzd8 (frizzled 4/8)	Impaired UB branching, renal hypoplasia		432
LGR4	Severe renal hypoplasia and oligonephronia; renal cysts	Aniridia–genitourinary anomalies–mental retardation syndrome	433, 434
LMX1B	Renal dysplasia, skeletal abnormalities	Nail-patella syndrome	334, 341
Mdm2 (murine double minute 2)	Renal hypoplasia and dysplasia, severely impaired UB branching and nephrogenesis (UB selective); depletion of nephrogenic precursors (MM selective)		435, 436
Mf2	Renal hypoplasia, oligonephronia		437
Pbx1	Reduced UB branching, delayed mesenchyme-to-epithelial transformation, dysgenesis of adrenal gland and gonads		438, 439
Plxnb2 (plexin B2)	Renal hypoplasia and ureter duplication		440
Pou3f3 (*Brn1*)	Impaired development of distal tubules, loop of Henle, and macula densa; distal nephron patterning defect		251
Prr (prorenin receptor)	Renal hypoplasia, renal dysplasia (UB selective)		441
Psen1, Psen2 (presenilins 1/2)	Severe renal hypoplasia, severe defects in nephrogenesis		247
Ptgs2 (prostaglandin endoperoxide synthase 2/ cyclooxygenase-2)	Oligonephronia		442
Rbpj	Severe renal hypoplasia, oligonephronia, loss of proximal nephron segments, tubular cysts (MM selective)		248, 249
Shp2	Severe impairment of UB branching, renal hypoplasia		163

Table 1.1 Summary of Knockout and Transgenic Models for Kidney Development (Continued)

Gene Mutation or Knockout	Renal and Urogenital Tract Phenotypes, Other Tissues Affected	Associated Human Disease(s)	Reference(s)
Six1	Hydronephrosis, hydroureter, abnormal development of ureteral smooth muscle		309
Six2	Renal hypoplasia, premature depletion of nephrogenic precursors		235
Tbx18	Hydronephrosis, hydroureter, abnormal development of ureteral smooth muscle		307, 309
Tfap2b	MM failure, craniofacial and skeletal defects		443
TRPS1	Impaired UB branching, renal hypoplasia	Trichorhinophalangeal syndrome (skeletal defects)	444
Wnt4	Failure of MM induction		223
Wnt7b	Complete absence of medulla and renal papilla (UB selective)		190
Wnt9b	Vestigial kidney, failure of MM induction Cystic kidney (collecting duct selective)		191, 220
Wnt11	Impaired ureteric branching, renal hypoplasia		165
Mislocalized or Ectopic UB/Increased UB Branching			
Bmp4, Bmp7	Ectopic UB, renal hypodysplasia, hydroureter, defective ureterovesical junction		218, 445
Cer1	Increased ureteric branching, altered spatial organization of ureteric branches		446
Foxc1	Duplex kidneys, ectopic ureters, hydronephrosis, hydroureter		214
HNF1B, PAX2	Renal hypoplasia, duplex kidneys, ectopic ureters, megaureter, hydronephrosis	CAKUT	447
Lzts2 (leucine-zipper putative tumor suppressor 2)	Duplex kidneys/ureters, hydronephrosis, hydroureter		448
Plxnb1 (plexin B1)	Increased ureteric branching		449
Plxnb2 (plexin B2)	Renal hypoplasia and ureter duplication		440
Sema3a	Increased ureteric branching (UB selective)		40
Slit2, ROBO2	Increased UB branching	CAKUT, VUR	215, 216
Spry1 (sprouty 1)	Supernumerary UBs, multiple ureters		70, 173
Cysts			
Aqp11 (aquaporin-11)	Abnormal vacuolization of proximal tubules; polycystic kidneys		450
Bcl2	Renal hypoplasia and cysts		451
Bicc1	Polycystic kidneys		452
Bpck/TMEM67	Polycystic kidneys, hydrocephalus	Meckel's syndrome (multicystic renal dysplasia, neural tube defects)	453
Erbb4	Renal cysts (overexpression in renal tubules) Dilated and mispolarized tubules, increased renal fibrosis (renal tubule deletion)		454
FAT4	Renal cysts, disrupted hair cell organization in inner ear	Van Maldergem's syndrome (mental retardation, abnormal craniofacial features, deafness, skeletal and limb malformations, renal hypoplasia)	259, 455

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Table 1.1 Summary of Knockout and Transgenic Models for Kidney Development (Continued)

Gene Mutation or Knockout	Renal and Urogenital Tract Phenotypes, Other Tissues Affected	Associated Human Disease(s)	Reference(s)
GLIS3	Polycystic kidney, neonatal diabetes	Congenital hypothyroidism, diabetes mellitus, hepatic fibrosis, congenital glaucoma	456, 457
GPC3 (glypican-3)	Disorganized tubules and medullary cysts	Simpson-Golabi-Behmel syndrome	458-460
HNF1B	Polycystic kidney disease (tubular-selective)	Maturity-onset diabetes of the young type 5 (MODY5)	260, 261
Ift88/Orpk (intraflagellar transport 88/Oak Ridge Polycystic Kidney Disease)	Polycystic kidneys; defective left-right asymmetric patterning		461, 462
Invs (inversin)	Polycystic kidneys, inverted viscera		463, 464
Kif3A	Polycystic kidney disease (tubular-selective)		465
MAFB (Kreisler)	Decreased glomeruli, cysts, and tubular dysgenesis	Musculoaponeurotic fibrosarcoma	466, 467
MKS1	Renal hypoplasia and cysts	Meckel's syndrome (multicystic renal dysplasia, neural tube defect)	468
PKD1, PKD2	Renal cysts	ADPKD, ARPKD	469
PTEN	Abnormal ureteric bud branching, cysts (UB selective)	Cowden's disease, Bannayan-Riley-Ruvalcaba syndrome, various tumors	164
Taz/Wwtr1	Polycystic kidneys, emphysema		470, 471
VHL	Renal cysts (tubular-selective)	Von Hippel–Lindau syndrome	472
Xylt2 (xylosyltransferase 2)	Polycystic kidneys and liver		473
Later Phenotypes (Glomerular, Vascular, and Glomerular Basement Membrane)			
ACE (angiotensin-converting enzyme)	Atrophy of renal papillae, vascular thickening and hypertrophy, perivascular inflammation	Chronic systemic hypotension	203, 204
ACTN4 (α-actinin 4)	Glomerular developmental defects, FSGS	SRNS	349, 350
AGT (angiotensinogen)	Atrophy of renal papillae, vascular thickening and hypertrophy, perivascular inflammation	Essential hypertension, renal tubular dysgenesis	205, 326
AGTR1A (AT1A)	Hypertrophy of juxtaglomerular apparatus and expansion of renin cell progenitors, mesangial cell hypertrophy	Essential hypertension, renal tubular dysgenesis	474
AGTR1A, AGTR1B (*AT1A, AT1B*)	Atrophy of renal papillae, vascular thickening and hypertrophy, perivascular inflammation	Essential hypertension, renal tubular dysgenesis	206
AMPD (AMP [adenosine monophosphate] deaminase)	Podocyte foot process effacement, proteinuria	Minimal change nephrotic disease	475
Angpt1/ANG1 (angiopoietin 1)	Simplification and dilation of glomerular capillaries; detachment of glomerular endothelium from the GBM; loss of mesangial cells		286
Angpt2/ANG2 (angiopoietin 2)	Cortical peritubular capillary abnormalities (null allele) Apoptosis of glomerular capillaries, proteinuria (transgenic overexpression)		295, 296
ARHGDIA/RhoGDIα	FSGS	SRNS	351, 352
Bmp7	Hypoplastic kidney, impaired maturation of nephron, reduced proximal tubules (podocyte selective)		302
CD151	Podocyte foot process effacement, disorganized GBM, tubular cystic dilation	Nephropathy (FSGS) associated with pretibial epidermolysis bullosa and deafness	476, 477

Table 1.1 Summary of Knockout and Transgenic Models for Kidney Development (Continued)

Gene Mutation or Knockout	Renal and Urogenital Tract Phenotypes, Other Tissues Affected	Associated Human Disease(s)	Reference(s)
CD2AP	Podocyte foot process effacement, immunotactoid nephropathy	FSGS	387
Cdc42	Congenital nephrosis; impaired formation of podocyte foot processes (podocyte selective)		374
Cmas	Congenital nephrosis; impaired formation of podocyte foot processes, defective sialylation		478
COL4A1, COL4A3, COL4A4, COL4A5	Disorganized GBM, proteinuria	Alport's syndrome	479-482
Crk1/2, CrkL	Albuminuria, altered podocyte cytoarchitecture (podocyte selective)		483
Cxcl12/SDF1 (stroma-derived factor 1), *CXCR4, Cxcr7*	Petechial hemorrhage in the kidneys, glomerular aneurysm, fewer glomerular fenestrations, reduced mesangial cells, podocyte foot process effacement, mild renal hypoplasia	WHIM (warts, hypogammaglobulinemia, infections, and myelokathexis) syndrome	305, 306, 484
DICER1	Podocyte damage, albuminuria, end-stage kidney failure (podocyte selective); reduced renin production, renal vascular abnormalities, striped fibrosis (renin cell selective)	Pleuropulmonary blastoma	329, 396-398
Dnm1, Dnm2 (dynamin 1/2)	Podocyte foot process effacement and proteinuria (podocyte selective)		485
EphB4	Aberrant development of vascular shunts in glomerular arterioles (transgenic overexpression in renal tubules and parietal cells of Bowman's capsule)		300
Ephrin-B2	Dilation of glomerular capillaries		301
Fat1	Foot process fusion, failure of foot process formation		353
Flt1/VEGFR1	Nephrotic syndrome		395
Foxc2	Impaired podocyte differentiation, dilated glomerular capillary loop, poor mesangial migration		64
Foxi1	Distal renal tubule acidosis; absence of collecting duct intercalated cells		255
Fyn	Podocyte foot process effacement, abnormal slit diaphragms, proteinuria		382, 486
Gne/Mnk (glucosamine-2-epimerae/*N*-acetylmannosamine kinase)	Hyposialylation defect, foot process effacement, GBM splitting, proteinuria and hematuria		487
Ilk (integrin-like kinase)	Nephrotic syndrome (podocyte selective)		384
INSR (insulin receptor)	Podocyte effacement, GBM alteration, proteinuria (podocyte selective)	Diabetic nephropathy	488
Itga3 (integrin α_3)	Reduced UB branching, glomerular defects, poor foot process formation		195, 197
Itgb1 (integrin β_1)	Podocyte loss, capillary and mesangial degeneration, glomerulosclerosis (podocyte selective)		410, 411

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Table 1.1 Summary of Knockout and Transgenic Models for Kidney Development (Continued)

Gene Mutation or Knockout	Renal and Urogenital Tract Phenotypes, Other Tissues Affected	Associated Human Disease(s)	Reference(s)
Kirrel (Neph1)	Abnormal slit diaphragm function, FSGS		63
Lama5	Defective glomerulogenesis, abnormal GBM, poor podocyte adhesion, loss of mesangial cells		186
LAMB2	Proteinuria prior to the onset of foot process effacement	Pierson's syndrome	187, 489
LMX1B	Impaired differentiation of podocytes, cytoskeletal disruption in podocytes	Nail-patella syndrome	490-492
Mafb (Kreisler)	Abnormal podocyte differentiation		335
Mpv17 (mitochondrial inner membrane protein 17)	Nephrotic syndrome		493
Mtor/mTOR (mechanistic target of rapamycin)	Proteinuria, podocyte autophagy defects (podocyte selective)		494
MYO1E	Podocyte foot process effacement and proteinuria	SRNS	354, 495, 496
Nck1, Nck2	Failure of foot process formation (podocyte selective)		380
Nid1 (nidogen-1/entactin-1)	Abnormal GBM		497
NPHS1 (nephrin)	Absent slit diaphragms, congenital nephrotic syndrome	Congenital nephrosis of the Finnish type, childhood-onset steroid-resistant nephritic syndrome, childhood- and adult-onset FSGS	342
NPHS2 (podocin)	Congenital nephrosis, FSGS, vascular defects	SRNS, congenital nephritic syndrome	343, 498
NOTCH1, NOTCH2	Lack of glomerular endothelial and mesangial cells (standard knockout) Lack of podocytes and proximal tubular cells (MM selective); impaired nephrogenesis (cap mesenchyme selective)	Alagille's syndrome (cholestatic liver disease, cardiac disease, kidney dysplasia, renal cysts, renal tubular acidosis)	243, 244, 248, 499
Pdgfb/PDGFR-β	Lack of mesangial cells, ballooned glomerular capillary loop		332, 333
Pik3c3/Vps34	FSGS, defects in vesicular trafficking (podocyte selective)		500, 501
Prkci/aPKCλ/ι (atypical protein kinase C λ/ι)	Defect of podocyte foot processes, nephrotic syndrome (podocyte selective)		375, 376
PTPRO/GLEPP1 (glomerular epithelial protein phosphatase 1)	Broadened podocyte foot processes with altered interdigitation patterns	SRNS	487, 502
Rab3A	Albuminuria, disorganization of podocyte foot process structure		15
Rbpj	Decreased renal arterioles, absence of mesangial cells, and depletion of renin cells (stromal cell selective) Reduction in juxtaglomerular cells, impaired renin synthesis (renin cell selective)		310, 331
Rhpn1 (rhophilin-1)	FSGS, podocyte foot process effacement, GBM thickening		355
ROBO2	Abnormal pattern of podocyte foot process interdigitation, focal effacement of foot processes, proteinuria	CAKUT, VUR	503
SLC5A2/SGLT2 (sodium-glucose transporter 2)	Elevated urinary excretion of glucose, calcium, and magnesium	Glucosuria	504
Sh3gl1/2/3 (endophilin 1/2/3)	Podocyte foot process effacement and proteinuria, neuronal defects		485

Table 1.1 Summary of Knockout and Transgenic Models for Kidney Development (Continued)

Gene Mutation or Knockout	Renal and Urogenital Tract Phenotypes, Other Tissues Affected	Associated Human Disease(s)	Reference(s)
Sirpa/SIRPα	Irregular podocyte foot process interdigitation; mild proteinuria		505
Sox4	Oligonephronia, podocyte effacement, GBM defects (MM selective)		506
SOX17, SOX18	Vascular insufficiency in kidneys and liver; ischemic atrophy of renal and hepatic parenchyma; defective postnatal angiogenesis	HLT (hypotrichosis-lymphedema-telangiectasia) syndrome (hair, vascular and lymphatic disorder)	311, 314
Synj1 (synaptojanin 1)	Podocyte foot process effacement and proteinuria; neuronal defects		485
Tcf21 (Pod1/capsulin/epicardin)	Lung and cardiac defects, sex reversal and gonadal dysgenesis, vascular defects, disruption in UB branching, impaired podocyte differentiation, dilated glomerular capillary, poor mesangial migration		6, 264
Tie1	*Tie1*–null cells fail to contribute to the glomerular endothelium		297
TRPC6	Protected from angiotensin-mediated or proteinuria or complement-dependent glomerular injury (null mutation); podocyte foot process effacement and proteinuria (transgenic overexpression in the podocyte lineage)	SRNS, FSGS	356, 393, 394, 507-510
Vegfa	Endotheliosis, disruption of glomerular filtration barrier formation, nephrotic syndrome (podocyte selective)		279, 280, 511
VHL	Rapidly progressive glomerulonephritis (podocyte selective)	Von Hippel–Lindau syndrome	304

AD, Autosomal dominant; AR, autosomal recessive; CAKUT, congenital anomalies of the kidney and urinary tract; CM, cap mesenchyme; FSGS, focal segmental glomerulosclerosis; GBM, glomerular basement membrane; MM, metanephric mesenchyme; PKD, polycystic kidney disease; SRNS, steroid-resistant nephrotic syndrome; UB, ureteric bud; VUR, vesicoureteral reflux.

heritability. Mutations may be complete or partial loss of function, gain of function, or altered function and can have either dominant or recessive effect. The specific locus mutation frequency of ENU is 1 in 1000. Assuming a total number of 25,000 to 40,000 genes in the mouse genome, a single treated male mouse should have between 25 and 40 different heterozygous mutagenized genes. In the case of multigenic phenotypes, segregation of the mutations in the next generation allows the researcher to focus on monogenic traits. In each generation, 50% of the mutations are lost, and only the mutation underlying the selected phenotype is maintained in the colony. A breeding strategy that includes backcrossing to the female genetic strain enables rapid mapping of the ENU mutation that occurred on the male genetic background.

The screening in ENU mutagenesis experiments can focus on dominant or recessive renal mutations. Screening for dominant phenotypes is popular because breeding schemes are simple and a great number of mutants can be recovered through this approach. About 2% of all first-generation offspring mice display a heritable phenotypic abnormality.[56,57] One of the fruitful results obtained with this approach is the identification of a mutation in the aquaporin 11 gene *(Aqp11)* that causes severe proximal tubule injury and vacuolation of the renal cortex resulting in renal failure and perinatal death.[58] It is possible to design "sensitized screens" on a smaller scale, thereby improving the ability to identify genes in a pathway of interest. For example, in renal glomerular development, the phenotype of a genetic mouse strain with a tendency to development of congenital nephrosis (e.g., *CD2AP* haploinsufficiency) may be enhanced or suppressed by breeding a female of the strain to a mutagenized male.[59] The modifier gene may then be mapped using the approach outlined earlier. This approach has been successfully used to identify genes involved in neural development.[60,61]

Table 1.2 Conditional Mouse Lines for the Kidney

Promoter	Renal Expression	Extrarenal Expression	Reference(s)
11Hsd2 (11β-hydroxysteroid dehydrogenase-2)	Principal cells of collecting duct, connecting tubules	Amygdala, cerebellum, colon, ovary, uterus, epididymis, salivary glands	512
Aqp2 (aquaporin-2)	Principal cells of collecting duct	Testis, vas deferens	513
Atp6v1b1 (V-ATPase-B1)	Collecting ducts (intercalated cells), connecting tubule		514, 515
Bmp7	Cap mesenchyme		516
Cdh16/Ksp-cadherin	Renal tubules, collecting ducts, ureteric bud, wolffian duct, mesonephros	Müllerian duct	75, 517
Cited1	Cap mesenchyme		18
Emx1	Renal tubules (proximal and distal tubules)	Cerebral cortex, thymus	518
Foxd1/BF2	Stromal cells		519
Ggt1 (gamma-glutamyl transferase 1)	Cortical tubules		520
HoxB6	Metanephric mesenchyme	Lateral mesoderm, limb buds	409, 521
HoxB7	Ureteric bud, wolffian duct, collecting ducts, distal ureter	Spinal cord, dorsal root ganglia	209
Kap (kidney androgen regulated protein)	Proximal tubules	Brain	522
Klf3	Collecting ducts	Gonads	541
Nphs1 (nephrin)	Podocytes	Brain	523, 524
Nphs2 (podocin)	Podocytes		525
Osr2	Condensing metanephric mesenchyme; glomeruli	Palatal mesenchyme	526
Pax2	Pronephric duct, wolffian duct, ureteric bud, cap mesenchyme	Inner ear, midbrain, cerebellum, olfactory bulb	527
Pax3	Metanephric mesenchyme	Neural tube, neural crest	525, 528, 529
Pax8	Renal tubules (proximal and distal tubules) and collecting ducts (Tet-On inducible system)		530
Pdgfrb (PDGFR-β)	Mesangial cells, vascular smooth muscles	Pericytes, vascular smooth muscles	301, 531
Pepck	Proximal tubules	Liver	472
Rarb2	Metanephric mesenchyme		412
Ren1 (Renin)	Juxtaglomerular cells, afferent arterioles, mesangial cells	Adrenal gland, testis, sympathetic ganglia, etc.	319
Ret	Ureteric bud, collecting ducts	Dorsal root ganglion, neural crest	532
Sall1	Metanephric mesenchyme (tamoxifen-inducible system)	Limb buds, central nervous system, heart	533
Slc5a2/SGLT2 (sodium-glucose transporter 2)	Proximal tubules		534
Six2	Cap mesenchyme		19
Sox18	Cortical and medullary vasculature	Blood vessel and precursor of lymphatic endothelial cells	535-537
Spink3	Medullary tubules (distal or connecting tubules?)	Mesonephric tubules, pancreas, lung, liver, gastrointestinal tract	528, 529, 538
T (brachyury)	Whole kidney (both ureteric bud and metanephric mesenchyme)	Panmesodermal	428
Tcf21 (Pod1)	Metanephric mesenchyme, cap mesenchyme, podocytes, stromal cells	Epicardium, lung mesenchyme, gonad, spleen, adrenal gland	193
Umod (uromodulin/Tamm-Horsfall protein)	Thick ascending loops of Henle	Testis, brain	539
Wnt4	Renal vesicles, nascent nephrons (comma- and S-shaped bodies)		19, 540

Other genomewide approaches that have led to the discovery of novel genes in kidney development and disease are gene trap consortia[62,63] and genomewide transcriptome and proteome projects.[64-66] The interested reader is referred to the websites for the Centre for Modeling Human Disease (www.cmhd.ca), the International Gene Trap Consortium (www.genetrap.org), Knockout Resources to Conquer Human Disease (www.tigm.org), and the Human Kidney & Urine Proteome Project (www.hkupp.org).

IMAGING AND LINEAGE TRACING STUDIES

Detailed imaging of renal structures and morphogenetic processes has benefited significantly from the availability and development of multiple fluorescent proteins. The advent of genetically modified mice that express fluorescent proteins revolutionized cell lineage and mapping studies allowing high-resolution live visualization of morphogenetic events both in situ and in cultured organ explants. Targeted labeling of cells with fluorescent proteins can be achieved by driving expression of fluorescent proteins under direct control of a cell-specific promoter. Alternatively, a Cre driver mouse can be crossed with a fluorescent reporter animal, whereby Cre recombinase (an enzyme that triggers swapping, or recombination, of stretches of DNA in chromosomes) turns on the constitutive expression of a fluorescent protein. This Cre-driven strategy is particularly valuable in cell lineage tracking and fate mapping analysis because both the progenitor and its subsequent derivatives become fluorescently labeled. A third method involves spatiotemporal induction of fluorescent protein expression, allowing for the fluorescence to be turned on or off through administration of doxycycline or tamoxifen by either the tetracycline (Tet)- or estrogen receptor (ERT2)–dependent inducible system, respectively. This third method allows for the incomplete and pulse labeling of certain cell lineages, permitting the tracking of the fate and migratory behavior of individual cells in real time.

HoxB7-EGFP is the first fluorescent transgene developed to visualize renal development.[67] Enhanced green fluorescent protein (EGFP), placed under the control of the *HoxB7* promoter, specifically labels the wolffian duct and the ureteric epithelial lineage. *HoxB7-EGFP* has therefore proved to be invaluable in studying the rates and pattern of ureteric branching morphogenesis and ureteral development, including disruption of these events in the context of particular mutant backgrounds.[68-71] The *HoxB7–myr-Venus* transgene, designed to express a membrane-bound myristoylated variant of EGFP (myr-Venus), allows for the visualization of individual ureteric epithelial cells by confocal microscopy, thus facilitating observation of changes in cell shape and position.[72] Other fluorescent transgenes for imaging of ureteric epithelia are *Ret-EGFP* and *Ksp-cadherin* (*Cdh16-EGFP*). In *Ret-EGFP* mice, EGFP expression is most prominent in the ampullary tips of the UB.[73,74] In contrast, fluorophore expression is restricted in the UB trunk and stalk, and absent in the UB tips, in *Cdh16-EGFP* mice.[75] An ingenious strategy involving the creation of chimeric animals with wild-type epithelial cells expressing *HoxB7-EGFP* that are intermingled with cells derived from mutant ES cells engineered to express CFP (cyan fluorescent protein) under the control of *HoxB7-Cre* unraveled the distinctive dependence on genes such as *Ret, Etv4, Etv5,* and *Spry1* in the cellular sorting and rearrangement needed for ureteric branching (Figure 1.11).[76,77] Inducible transgene expression systems can be very useful in labeling a small subset of cells to enable the fate of the cells to be monitored temporally. A tamoxifen-inducible strategy to mark ureteric epithelial cells with myr-Venus has been cleverly used to observe the unique manner in which proliferating UB cells delaminate into the UB lumen and reposition themselves within the expanding UB ampullary tip.[78]

Lgr5-EGFP, Cited1-EGFP, and a variety of *Six2-EGFP* transgenes have been employed to characterize the self-renewing capacity and multipotency of nephron progenitor cells within the cap mesenchyme.[18,19,79] The mechanism by which nephrogenic and ureteric epithelia are physically conjoined via the invasion of the UB tip by distal nephron precursors has been imaged through the targeted expression of myr-Venus under the control of a *Six2-Cre* driver.[16] A wide variety of fluorescent protein transgenes and Cre transgenes are now available to characterize the development and organization of multiple compartments of the kidney (see Table 1.2).[80]

NONMAMMALIAN MODEL SYSTEMS FOR KIDNEY DEVELOPMENT

Organisms separated by millions of years of evolution from humans still provide useful models to study the genetic basis and function of mammalian kidney development. This continuing feature stems from the facts that all of these organisms possess excretory organs designed to remove metabolic wastes from the body and that genetic pathways involved in other aspects of invertebrate development may serve as templates to dissect pathways in mammalian kidney development. In support of the latter argument, elucidation of the genetic interactions and molecular mechanism of the Neph1 ortholog and nephrin-like molecules SYG-1 and SYG-2 in synapse formation in the soil nematode *Caenorhabditis elegans* is providing major clues to the function of their corresponding genes in glomerular and slit diaphragm formation and function in mammals.[81]

The excretory organs of invertebrates, which differ greatly in their structure and complexity, range in size from a few cells in *C. elegans* to several hundred cells in the malpighian tubules of the fly *Drosophila* to the more recognizable kidneys in amphibians, birds, and mammals. In *C. elegans*, the excretory system consists of a single large H-shaped excretory cell, a pore cell, a duct cell, and a gland cell.[82,83] *C. elegans* provides many benefits as a model system: the availability of powerful genetic tools including "mutants by mail," short life and reproductive cycle, publicly available genome sequence and resource database (www.wormbase.org), the ease of performing genetic enhancer-suppressor screens in worms, and the fact that they share many genetic pathways with mammals. Major contributions to our understanding of the function of polycystic and cilia-related genes have been made from studying *C. elegans*. The *PKD1* and *PKD2* homologs in *C. elegans, lov-1* and *lov-2,* are involved in cilia development and function of the mating organ required for mating behavior.[84,85] Strides in understanding the function of the slit diaphragm have also been made from studies of *C. elegans,* as described earlier.

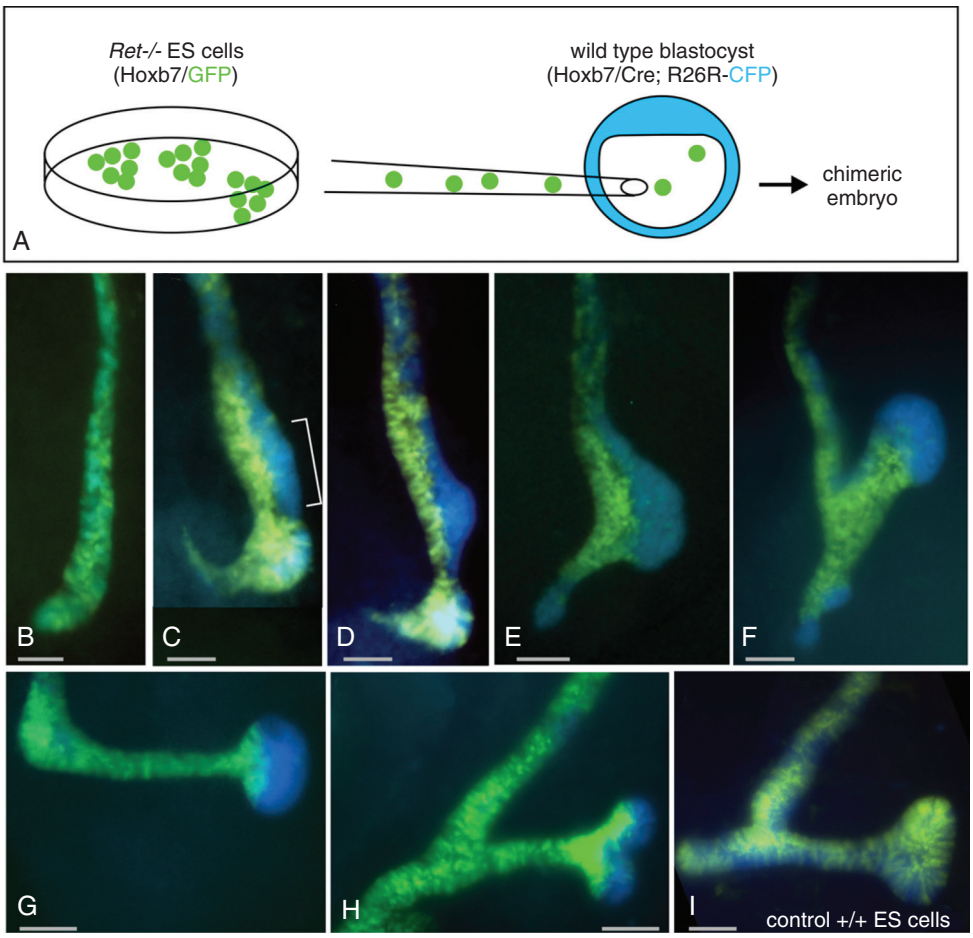

Figure 1.11 Cell fate tracing through genetic expression of fluorophores. Segregation of Ret-deficient cells in the outgrowth and branching of the ureteric bud (UB). **A,** Ret-null embryonic stem cells (ES) expressing HoxB7-GFP (green fluorescent protein) were mixed with a wild-type transgenic blastocyst (HoxB7-Cre: R26R-CFP [cyan fluorescent protein]). This process generates chimeric animals in which *Ret*-null cells exhibit GFP fluorescence and wild-type UB cells express CFP. **B,** At 9.5 dpc (days post coitum), *Ret*-null epithelial cells are intermingled with wild-type cells in the wolffian duct (WD). **C,** At 10 dpc, when the dorsal side of the WD begins to swell, the region where the UB will emerge becomes enriched with CFP-expressing but not *Ret*-null cells. **D** and **E,** At around 10.5 dpc, the UB is formed exclusively by wild-type cells. **F,** Upon elongation of the UB at 11 dpc, the bulbous distal tip of the UB is formed by wild-type cells but the *Ret*-null cells begin to contribute to the trailing trunk structure. **G** and **H,** During the initial branching of the UB at around 11.5 dpc, *Ret*-null cells are excluded from the distal ampullary UB tips. **I,** In contrast, control cells expressing Ret and GFP contribute to the whole branching UB structure. (Reproduced with permission from Chi X, Michos O, Shakya R, et al: Ret-dependent cell rearrangements in the Wolffian duct epithelium initiate ureteric bud morphogenesis. *Dev Cell* 17:199-209, 2009.)

In *Drosophila*, the "kidney" consists of malpighian tubules that develop from the hindgut and perform a combination of secretory, resorption, and filtering functions.[86] They express a number of mammalian gene homologs (e.g., *Cut,* members of the Wingless pathway) that have subsequently been shown to play major roles in mammalian kidney development. Furthermore, studies on myoblast fusion and neural development in *Drosophila*—two processes that may not appear to be related to kidney development at first glance—have provided major clues to the development and function of slit diaphragms.[87] Mutations in the fly *Neph* ortholog, irregular chiasm C-roughest (*irreC-rst*), are associated with neuronal defects and abnormal patterning of the eye.[88,89]

The pronephros, which is only the first of three stages of kidney development in mammals, is the final and only kidney of jawless fishes, whereas the mesonephros is the definitive kidney in amphibians. The pronephros found in larval stage zebrafish (*Dario rerio*) consists of two tubules connected to a fused, single, midline glomerulus. The zebrafish pronephric glomerulus expresses many of the same genes found in mammalian glomeruli (e.g., *Vegfa, Nphs1, Nphs2,* and *Wt1*) and contains podocytes and fenestrated endothelial cells.[90] Advantages of the zebrafish as a model system include its short reproductive cycle, transparency of the larvae with easy visualization of defects in pronephric development without sacrifice of the organism, availability of the genome sequence, the ability to rapidly knock down gene function with morpholino oligonucleotides, and the ability to perform functional studies of filtration using fluorescently tagged labels of varying sizes.[91] These features make zebrafish amenable to both forward and reverse genetic screens. Currently, multiple laboratories perform knockdown screens of mammalian homologs and

genomewide mutagenesis screens in zebrafish in order to study renal function.

The pronephros of the clawed frog *Xenopus laevis* has also been used as a simple model to study early events in nephrogenesis. As in the fish, the pronephros consists of a single glomus, paired tubules, and a duct. The fact that *X. laevis* embryos develop rapidly outside the body (all major organ systems are formed by 6 days of age), the ease of injecting DNA, messenger RNA, and protein, and the ability to perform grafting and in vitro culture experiments establish the frog as a valuable model system for dissection of early inductive and patterning cues.[92] In addition, insights emerging from the use of the chick embryo as a model for mesonephros development have highlighted the role of the Vg1/Nodal signaling pathway in formation of the intermediate mesoderm as the embryonic source of all kidney tissue in vertebrates.[93]

GENETIC ANALYSIS OF MAMMALIAN KIDNEY DEVELOPMENT

Much has been learned about the molecular genetic basis of kidney development over the past 15 years. This understanding has been gained primarily through the phenotypic analysis of mice carrying targeted mutations that affect kidney development. Additional information has been gained by identification and study of genes expressed in the developing kidney, even though the targeted mutation, or knockout, either has not yet been performed or has not affected kidney development or function. This section categorizes the genetic defects on the basis of the major phenotype and stage of disrupted development. It must be emphasized that many genes are expressed at multiple points of renal development and may play pleiotropic roles that are not entirely clear.

INTERACTION OF THE URETERIC BUD AND THE METANEPHRIC MESENCHYME

The molecular analysis of the initiation of metanephric kidney development has included a series of classic experiments using organ culture systems that allow separation of the UB and the MM as well as a later analysis of many gene-targeted mice with phenotypes that included various degrees of renal agenesis. As previously mentioned, the organ culture system has been in use since the seminal experiments, beginning in the 1950s, of Grobstein, Saxen, and their colleagues.[27,94,95] These experiments showed that the induction of the mesenchymal-to-epithelial transformation within the MM required the presence of an inducing agent provided by the UB. The embryonic neural tube was found to be able to substitute for the epithelial bud, and experiments involving the placement of the inducing agent on the opposite side of a porous filter from the mesenchyme provided information about the degree of contact required between them. A large series of experiments using organ cultures provided information about the timing of appearance of different proteins normally observed during the induction of nephrons and about the intervals that were crucial in maintaining contact between the inducing agent and the mesenchyme to obtain induction of tubules.

The work with the organ culture system provided an extensive framework on which to base further studies of organ development, and the system remains in extensive use to this day. However, the modern era of studies on the early development of the kidney began with the observation of renal agenesis phenotypes in gene-targeted or knockout mice, the earliest among these being the knockout of several transcription factors, including the WT1, Pax2, Eya1, Osr1/Odd1, Six1, Sall1, Lhx1/Lim1, and Emx2.[28,29,37,96-101] The knockout of several secreted signaling molecules, such as GDNF, GDF11, gremlin (Grem1), and the receptors Ret and GFRα1 (GDNF family receptor alpha1), also resulted in renal agenesis, at least in the majority of embryos.[102-108]

EARLY LINEAGE DETERMINATION OF THE METANEPHRIC MESENCHYME

In most embryos exhibiting renal agenesis, an appropriately localized putative MM is often uninvaded by a UB outgrowth. Two exceptions are the *Osr1/Odd1* and *Eya1* mutant embryos, in which this distinct patch of MM is absent, suggesting that *Osr1* and *Eya1* represent the earliest determinants of the MM yet identified (Figure 1.12). Together, the phenotypes of these knockout mice have provided an initial molecular hierarchy of early kidney development.[96,109] *Osr1* is localized to mesenchymal cells within the mesonephric and metanephric kidney and is subsequently downregulated upon epithelial differentiation. Mice lacking *Osr1* do not form the MM and do not express several other factors required for metanephric kidney formation, including Eya1, Six2, Pax2, Sall1, and GDNF.[109] Other factors implicated in the earliest stages of MM cell fate determination are the Eya1/Six1 pathway. *Eya1* and *Six1* mutations are found in humans with branchiootorenal (BOR) syndrome.[110] It is now known through in vitro experiments that the proteins Eya1 and Six1 form a regulatory complex that appears to be involved in transcriptional regulation.[111,112] Interestingly, Eya1 was shown to have an intrinsic phosphatase activity that regulates the activation of the Eya1/Six1 complex.[112,113] Moreover, Eya and Six family genes are co-expressed in several tissues in mammals, *Xenopus,* and *Drosophila*, further supporting a functional interaction between these genes.[96,100,101,114,115] Direct transcriptional targets of this complex appear to include the pro-proliferative factor c-Myc.[112] In the *Eya1*-deficient urogenital ridge the putative MM is completely absent.[116] Consistent with this finding, Six1 is either absent or poorly expressed in the presumptive location of the MM of *Eya1*-null embryos.[112,114–116] These findings may identify *Eya1* as a gene involved in early commitment of this group of cells to the metanephric lineage. Although Six1 and Eya1 may act in a complex together, the Six1 phenotype is somewhat different, in that a histologically distinct mesenchyme is present at 11.5 dpc, without an invading UB, similar to the other renal agenesis phenotypes.[100,101] Eya1 is expressed in the *Six1*-null mesenchyme, suggesting that *Eya1* is upstream of *Six1.* Additionally, Sall1 and Pax2 are not expressed in the *Six1* mutant mesenchyme even though WT1 is expressed.[100,101,116] There are discrepancies in the literature about Pax2 expression in *Six1* mutant embryos, which may reflect the exact position along the anterior-posterior axis of the urogenital ridges of *Six1* mutant embryos from which sections are obtained.

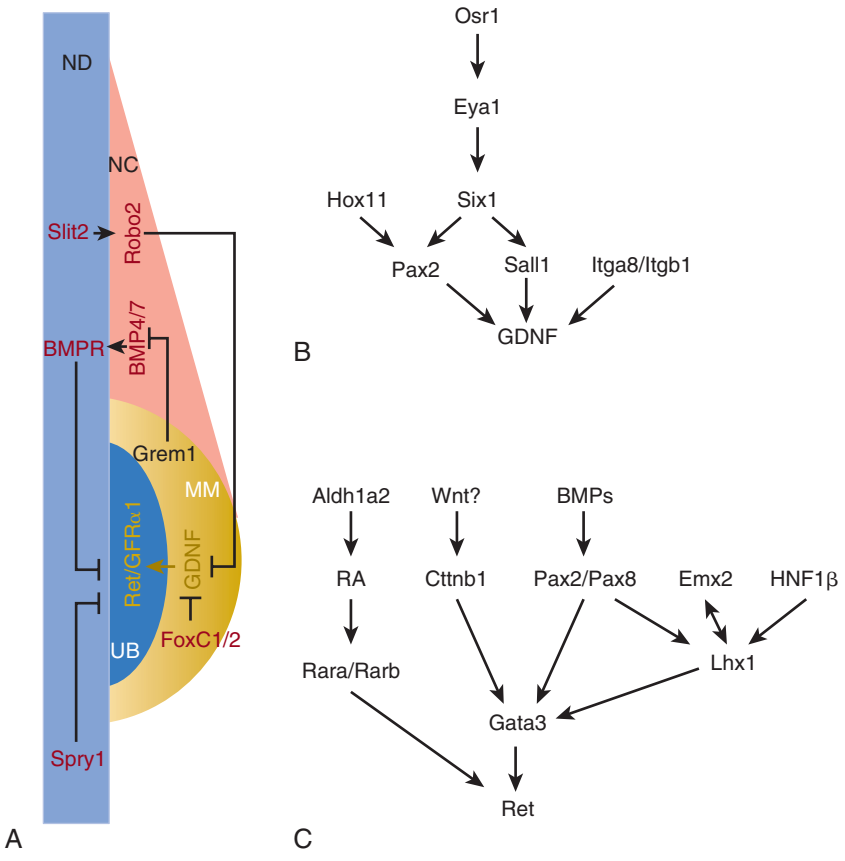

Figure 1.12 Genetic interactions during early metanephric kidney development. A, Regulatory interactions that control the strategically localized expression of GDNF (glial cell–derived neurotrophic factor) and Ret and the subsequent induction of the ureteric bud (UB). The anterior part of GDNF expression is restricted by Foxc1/2 and Slit/Robo2 signaling. Spry1 suppresses the post-receptor activity of Ret. BMP4/7-BMPR (bone morphogenetic protein 4/7–bone morphogenetic protein receptor) signaling inhibits the response to GDNF, an effect counteracted by gremlin 1 (Grem1). **B** and **C,** Genetic regulatory networks that control the expression of **(B)** GDNF and **(C)** Ret. MM, Metanephric mesenchyme; NC, nephrogenic cord; ND, nephric duct.

URETERIC BUD INDUCTION: TRANSCRIPTIONAL REGULATION OF GDNF

In many cases of renal agenesis, a failure of the GDNF-Ret signaling axis has been identified.[117] GDNF, a member of the tumor growth factor-β (TGF-β) superfamily and secreted by the MM, activates the Ret-GFRα1 receptor complex that is expressed by cells of the nephric duct and the UB. Activation of the Ret tyrosine kinase is of central importance to UB induction. Most mutant embryos lacking Gdnf, Ret, or Gfrα1 exhibit partial or complete renal agenesis owing to severe impairment of UB induction, whereas exogenous GDNF is suffice to induce sprouting of ectopic buds from the nephric duct.[103-106,118-121] Consistently, other genes linked to renal agenesis are known to regulate the normal expression of GDNF. These include genes encoding for transcription factors (e.g., Eya1, Pax2, Six1, Hox11 paralogs, and Sall1) and proteins required to stimulate or maintain GDNF expression (e.g., GDF11, Kif26b, nephronectin, $\alpha_8\beta_1$-integrin, and Fras1) (see Figure 1.12).[96,99,101,102,122-130]

As described earlier, *Eya1* mutants fail to form the MM. Pax2, a transcriptional regulator of the paired box *(Pax)* gene family, is expressed widely during the development of both UB and mesenchymal components of the urogenital system.[127] In *Pax2*-null embryos, Eya1, Six1, and Sal11 are expressed,[116] suggesting that the Eya1/Six1 is likely upstream of Pax2. Through a combination of molecular and in vivo studies, it has been demonstrated that Pax2 appears to act as a transcriptional activator of GDNF and regulates the expression of Ret.[128,131] Pax2 also appears to regulate kidney formation through epigenetic control because it is involved in the assembly of a histone H3, lysine 4 methyltansferase complex through the ubiquitously expressed nuclear factor PTIP (pax transcription activation domain interacting protein), which regulates histone methylation.[132] The *Hox* genes are conserved in all metazoans and specify positional information along the body axis. *Hox11* paralogs include *Hoxa11, Hoxc11,* and *Hoxd11.* Mice carrying mutations in any one of these genes do not have kidney abnormalities; however, triple-mutant mice for these genes demonstrate a complete absence of metanephric kidney induction.[129] Interestingly, in these mutants, the formation of condensing MM and the expressions of Eya1, Pax2, and WT1 remain unperturbed, suggesting that *Hox11* is not upstream of these factors. Although there seems to be some hierarchy, Eya1, Pax2, and Hox11 appear to form a complex to coordinately regulate the expression of GDNF.[133]

Sall1 indirectly controls the expression of GDNF. Sall1 is necessary for the expression of the kinesin Kif26b by the MM cells.[125] In the absence of either Sall1 or Kif26b, the nephronectin receptor $\alpha_8\beta_1$-integrin expressed by the MM mesenchyme is downregulated. The loss of Sall1, Kif26b, $\alpha_8\beta_1$-integrin, and nephronectin compromises the adhesion of the MM cells to the UB tips, ultimately causing loss of GDNF expression and failure of UB outgrowth.[124,126,134,] Loss of the extracellular matrix protein Fras1—the gene which is linked to Fraser's syndrome and which is expressed selectively in the UB epithelium and nascent epithelialized nephrons but not the MM—causes loss of GDNF expression.[122] Fras1 likely regulates MM induction and GDNF expression via multiple signaling pathways. Fras1 deficiency results in downregulation of GDF11, Hox11, Six2, and

α_8-integrin, and an increase in bone morphogenetic protein 4 (BMP4), which cooperatively controls GDNF expression.[122]

NON-GDNF PATHWAYS IN THE METANEPHRIC MESENCHYME

Another pathway in early development of the MM involves WT1 and vascular endothelial growth factor A (VEGF-A).[49] Induction of the UB does not occur in *Wt1* mutants, although GDNF is expressed in the MM, indicating the existence of a GDNF-independent UB induction mechanism.[28] However, details of this pathway still remain to be clarified. A novel approach to the organ culture system involving microinjection and electroporation has also yielded insights as to a possible function of the *Wt1* gene in early kidney development. Overexpression of WT1 from an expression construct led to high-level expression of VEGF-A. The target of VEGF-A appeared to be Flk1 (VEGF receptor 2 [VEGFR2])–expressing angioblasts at the periphery of the mesenchyme. Blocking signaling through Flk1, if done when the metanephric rudiment was placed in culture, blocked expression of Pax2 and GDNF and, consequently, of the continued branching of the UB and induction of nephrons by the bud. Blockade of Flk1 after the organ had been in culture for 48 hours had no effect, indicating that the angioblast-derived signal was required to initiate kidney development but not to maintain continued development.[49] The signal provided by the angioblasts is not yet known, nor is it known whether WT1 is a direct transcriptional activator of VEGF-A. Flk1 signaling is also required to initiate hepatocyte differentiation during liver development. Numerous targets of WT1 in nephron progenitors have been identified though chromatin immunoprecipitation, providing a comprehensive catalog of genes particularly enriched for functions relating to transcription, multiorgan development, and cell cycle regulation. In addition, a number of these WT1 targets have special roles in remodeling of the actin cytoskeleton.[38]

GENES REQUIRED BY THE URETERIC BUD IN EARLY KIDNEY DEVELOPMENT

Several components of the genetic network supporting the development of the nephric duct and the UB have been identified (see Figure 1.12). *Pax2* and *Pax8* are required to maintain the expression of *Lhx1*.[135] *Pax2, Pax8,* and *Lhx1* altogether likely coordinate the expression of *Gata3,* which is necessary for elongation of the nephric duct.[136] *Gata3* and *Emx2,* which are required for the expression of Ret in the nephric duct, are both regulated by β-catenin, an effector of the canonical Wnt signaling pathway (for a discussion of Wnt, see section "Molecular Analysis of the Nephrogenic Zone").[29,137,138] Acting likely in parallel with *Gata3* to maintain Ret expression in the UB is *Aldh1a2 (Raldh2),* a gene in the retinoic acid synthesis pathway.[139] Surprisingly, this genetic regulatory hierarchy cannot fully account for the distinctive phenotypes arising from the mutations of individual genes, suggesting that additional important components of the nephric duct genetic network have yet to be identified. Nephric duct specification fails in *Pax2/Pax8* mutants but not in the case of *Lhx1* deficiency, in which only the caudal portion of the nephric duct degenerates.[135] The absence of *Gata3* or *Raldh2* causes misguided elongation of the nephric duct, which terminates into either blind-ended ureters or abnormal connections between the bladder and urethra.[136] The curtailed caudal growth of the nephric duct when either *Lhx1* or *Gata3* is lost prevents the formation of the first UB and consequently causes renal agenesis.[136,140,141] The absence of *Aldh1a2* leads to the formation of ectopic ureters and hydronephrotic kidneys.[139] *Emx2* deficiency does not prevent caudal extension of the nephric duct toward the presumptive MM, but the evagination of the UB is aborted, thereby resulting in renal agenesis.[29] Without β-catenin, nephric duct cells undergo precocious differentiation into collecting duct epithelia.[142] Ret does not affect the nephric duct fate but has importance in later UB development and insertion of the nephric duct into the cloaca.[77,120,139] Identification of additional targets of Pax2, Pax8, Lhx1, Gata3, and β-catenin are necessary in order to fully understand these seemingly disparate mutant phenotypes.

UB induction and subsequent branching require a unique spatial organization of Ret signaling. The bulbous UB tip is a region enriched with proliferative ureteric epithelial cells, in contrast to the emerging stalk regions of the developing ureteric tree.[30,143] It is now well appreciated that receptor tyrosine kinase (RTK) signaling primarily through Ret is key to the proliferation of UB tip epithelia. Exogenous GDNF supplemented in explanted embryonic kidneys can cause expansion of the UB tip region toward the source of the ligand.[143-145] Erk kinase activation is prominent within the ampullary UB terminals, where Ret expression is elevated.[30] Consistently, chimera analysis in mice reveals that Ret-deficient cells do not contribute to the formation of the UB tips.[120] All together, these studies underscore the importance of strategic levels of Ret expression and activation of proliferative signaling pathways in the stereotypical sculpting of the nascent collecting duct network.

A ligand-receptor complex formed by GDNF, GFRα1, and Ret is necessary for autophosphorylation of Ret on its intracellular tyrosines (Figure 1.13). A number of downstream adaptor molecules and effectors have been identified to interact with active phosphorylated Ret, including the growth factor receptor–bound proteins Grb2, Grb7, and Grb10, ShcA, Frs2, phospholipase $C_{\gamma 1}$ ($PLC_{\gamma 1}$), Shp2, Src, and Dok adaptor family members (Dok4/5/6).[146-157] These downstream Ret effectors together are likely contributors to the activation of the Ras/SOS/Erk and PI3K/Akt pathways supporting the proliferation, survival, and migratory behavior of the UB epithelium.[30,32,158] Knock-in mutations of the interaction site for Shc/Frs2/Dok adaptors on the short isoform of Ret lead to the formation of rudimentary kidneys.[159-162] Specific mutation of the $PLC_{\gamma 1}$ docking site on Ret leads to renal dysplasia and ureter duplications.[159] The loss of Shp2 in the UB lineage also causes severe renal hypoplasia, phenocopying that is observed in occasional Ret-deficient kidneys.[163] UB-specific inactivation of *Pten*, a target of the PI3K/Akt pathway, disrupts UB branching.[164] Taken together, these findings underscore the significance of Ret signaling in normal UB branching.

A number of transcriptional targets of Ret activation in microdissected UB stimulated with GDNF have been elucidated (see Figure 1.13).[76] Among these are Ret itself and Wnt11, which stimulates GDNF expression in the MM,[165] suggesting that a positive feedback loop exists for the GDNF-Ret signaling pathway. Ret activation also positively regulates

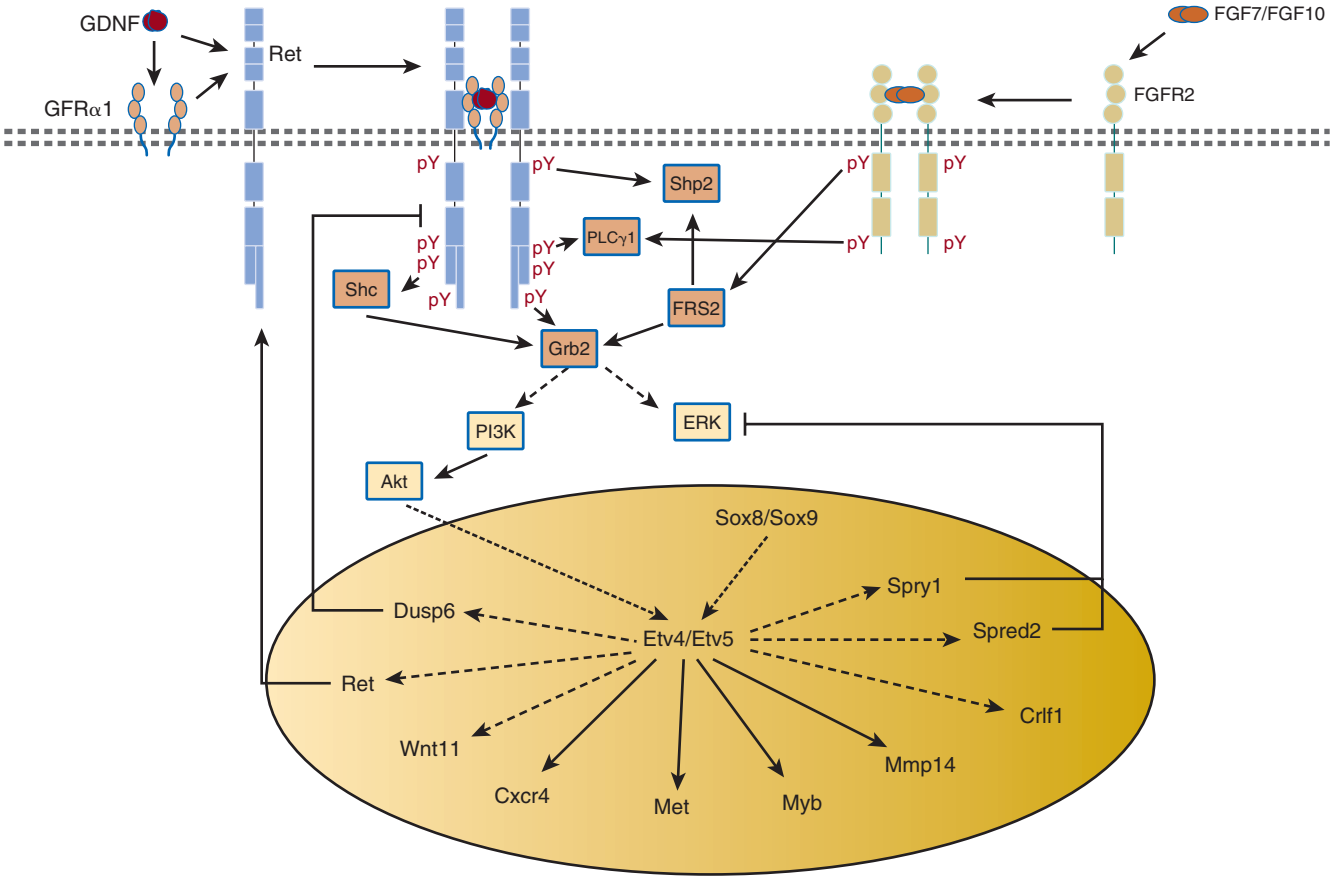

Figure 1.13 Ret signaling pathway. Ret is activated and becomes autophosphorylated on intracellular tyrosine residues (pY) upon association with GDNF GDNF (glial cell–derived neurotrophic factor) and its receptor GFRα1. Signaling molecules such as Grb2, Shc, FRS2, PLCγ1, and Shp2 bind directly to the phosphorylated tyrosine residues within the intracellular domain of Ret. Recruitment of Shc, FRS2, and Grb2 leads to activation of the ERK and PI3K/Akt pathways. GDNF-Ret signaling leads to the specific activation of a host of genes, some of which strongly depend on the upregulation of the transcription factors Etv4 and Etv5 *(solid arrows)*. Etv4/Etv5 activation requires activation of the PI3K/Akt but not the ERK pathway. Transcription factors Sox8 and Sox9 are believed to act in parallel to reinforce transcriptional responses to GDNF-Ret engagement. Some of these pathways are shared with the FGF7/10-FGFR2 receptor signaling system. The proteins Spry1 and Spred2 negatively regulate ERK signaling, whereas Dusp6 likely mitigates dephosphorylation of the Ret receptor, thus acting as part of a negative feedback regulatory loop. Other distinctive transcriptional targets of Ret activation include Crlf1, Cxcr4, Mmp14, Myb and Wnt11.

the ETS (E26 transformation-specific) transcription factors Etv4 and Etv5, which are also necessary for normal UB branching morphogenesis. *Etv4*-null homozygous mutants and compound heterozygous mutants for *Etv4* and *Etv5* manifest severe renal hypoplasia or renal agenesis, suggesting that these transcription factors are indispensable targets of Ret for proper UB development.[76] In chimeric animals Etv4/Etv5-deficient cells, just like Ret-deficient cells, fail to integrate within the UB tip domain.[120,166]

The gene *Sprouty* was identified as a general antagonist of RTKs and was discovered for inhibiting the fibroplastic growth factor (FGF) and epidermal growth factor (EGF) signaling pathways that pattern the *Drosophila* airways, wings, and ovarian follicles.[167-169] Of the four mammalian *Sprouty* homologs, *Spry1, Spry2,* and *Spry4* are expressed in developing kidneys.[170] *Spry1* is expressed strongly at the UB tips, whereas *Spry2* and *Spry4* are found in both the UB and the MM.[171] Sprouty molecules are thought to uncouple receptor tyrosine kinases with the activation of ERK pathway either through competitive binding with the Grb2/SOS complex or through the kinase Raf, effectively repressing ERK activation. Interestingly, *Spry1* expression is distinctively upregulated upon GDNF activation of Ret.[76] This finding suggests that Ret activates a negative feedback mechanism via Spry1 in order to control activated ERK levels and modulate cell proliferation in the UB. Studies on *Spry1*-knockout mice reveal some intriguing facets about Ret dependence of UB induction and branching.[70,72,172-175] *Spry1* deficiency leads to ectopic UB induction and can rescue renal development in the absence of either GDNF or Ret.[172,176] Germline inactivation of *Spry2* does not overtly affect renal development but can rescue renal hypoplasia in mice engineered to express *Ret* mutants impaired in activating the Ras/ERK pathway.[171] The transcriptional targets of Ret, such as Etv4, Etv5 and Wnt11, are retained in *Gdnf/Spry1* or *Ret/Spry1* compound null mutants.[172,176] These findings indicate that Ret signaling is not absolutely required for UB development. In fact, signaling via FGF10 and the receptor FGFR2 is sufficient for renal development despite the absence of GDNF or Ret, provided that *Spry1* is inactivated. Nevertheless, patterns of renal branching are distinctively altered in *Gdnf/Spry1* and *Gdnf/Ret* compound mutants, with UB tips

often displaying more heterogeneous shapes and orientation. These findings indicate that there remain some distinctive roles of GDNF-Ret signaling that cannot be fully compensated by FGF10/FGFR2 during UB development.

ADHESION PROTEINS IN EARLY KIDNEY DEVELOPMENT

A current theme in cell biology is that growth factor signaling often occurs coordinately with signals from the extracellular matrix transduced by adhesion receptors, such as members of the integrin family. $\alpha_8\beta_1$-integrin is expressed by cells of the MM interacting with the novel ligand nephronectin expressed specifically by UB cells.[124,177] In most embryos with mutations causing absence of α_8-integrin, UB outgrowth is arrested upon contact with the MM.[124] In a small portion of embryos, this block is overcome, and a single, usually hypoplastic, kidney develops. Nephronectin gene *(Npnt)* knockout mice exhibit renal agenesis or severe hypoplasia.[126] Thus, the interaction of $\alpha_8\beta_1$-integrin with nephronectin must have an important role in the continued growth of the UB toward the MM. Phenotypes of both *Itga8* and *Npnt* knockout mice appear to result from a reduction in GDNF expression.[126] The attraction of the UB to the mesenchyme is also governed by the maintenance of proper cell-cell adhesion within mesenchymal cells. Kif26b, a kinesin specifically expressed in the MM, is important for tight condensation of mesenchymal cells.[125] Genetic inactivation of *Kif26b* results in renal agenesis due to impaired UB induction. In *Kif26b* mutant mice, the compact aggregation of mesenchymal cells is compromised, resulting in distinctive loss of polarized expression of α_8-integrin and severe downregulation of GDNF expression. Hence, dysregulation of mesenchymal cell adhesion causes the failure to attract and induce the ureteric epithelia.

Genetic evidence further shows that nephronectin localization at the basement membrane of the UB is critical for GDNF expression by the MM. Genetic inactivation of basement membrane proteins associated with Fraser's syndrome (Fras1, Frem1/Qbrick, and Frem2) leads to renal agenesis characterized by severe downregulation of GDNF expression.[122,123,178-181] On the basis of interaction of nephronectin with Fras1, Frem1/Qbrick, and Frem2, it has been proposed that the Fras1/Frem1/Frem2 ternary complex anchors nephronectin to the UB basement membrane, thus stabilizing engagement with $\alpha_8\beta_1$-integrin expressed by the MM (Figure 1.14).[179] Grip1, a PDZ domain protein known to interact with Fras1, is required to localize the Fras1/Frem1/Frem2 complex on the basal aspect of the UB epithelium.[182] *Grip1* mutations phenocopy Fraser's syndrome, including renal agenesis, thus further highlighting the importance of the strategic localization of nephronectin on the UB surface toward the opposing MM.[182-184]

The establishment of epithelial basement membranes during metanephric kidney development involves the stage-specific assembly of different laminin α and β subunits with a common laminin γ_1 subunit. The UB-specific inactivation of the gene *Lamc1,* which encodes for laminin γ_1, leads to impaired UB induction and branching, ultimately causing either renal agenesis or hypomorphic kidneys with water transport deficits.[185] *Lamc1* deficiency prevents formation of basement membranes, causing downregulation of both growth factor (GDNF/Ret, Wnt11, and FGF2)–based and integrin-based signaling. This fact is another example of how signaling through the extracellular matrix intersects with growth factor signaling to influence morphogenesis. The importance of basement membrane assembly in the development of other renal structures is emphasized by genetic studies on the genes *Lama5* and *Lamb2,* which encode for laminins α_5 and β_2, respectively. Loss of *Lama5* causes either renal agenesis or disruption of glomerulogenesis, whereas deficiency of *Lamb2* leads to a defective glomerular filtration barrier.[186,187]

The UB branching program is stereotypically organized so that the proliferative UB epithelial cells are largely confined to the bulbous UB tips but cell division is dampened within the elongated nonbranching UB stalks of the growing ureteric tree. TROP2/Tacstd2, an adhesion molecule related to epithelial cell adhesion molecule (EpCAM), is expressed prominently in the UB stalks, where it colocalizes with collagen-1.[188] TROP2, unlike EpCAM, which is expressed throughout the UB tree, is not expressed at the UB ampullary tips. Consistently, dissociated and sorted UB cells expressing high levels of TROP2 are nonproliferative and express low levels of Ret, GFRα1, and Wnt11, which are notable UB tip markers. Elevated expression of TROP2 is also associated with poor attachment of epithelial cells to collagen matrix and with suppression of cell spreading and motility, thus emphasizing the importance of this adhesion molecule in negative regulation of UB branching and the sculpting of the nascent collecting duct network. The formation of patent lumens within epithelial tubules of the kidney also depends on coordinated cell adhesion. β_1-integrin is tethered to the actin cytoskeleton via a ternary complex formed between integrin-like kinase (ILK) and parvin. ILK has been shown to be important in mediating cell cycle arrest and cell contact inhibition in the collecting duct epithelia.[189] The targeted ablation of *Ilk* expression in the UB does not cause remarkable defects in UB branching but does eventually lead to postnatal death due to obstruction of collecting ducts arising from dysregulated intraluminal cell proliferation. Thus, cell adhesion molecules may suppress cell division to regulate distinctive aspects of renal branching and tubulogenesis.

FORMATION OF THE COLLECTING SYSTEM

The overall shape, structure, and size of the kidneys are largely guided by the stereotypical branching of the UB and the subsequent patterning of the collecting duct system. During late gestation, past embryonic stage 15.5 dpc in the mouse, the trunks of the UB tree undergo extensive elongation to establish the array of collecting ducts found in the renal medulla and papilla. The radial arrangement of elongated collecting ducts together with the loops of Henle (derived from the nephrogenic mesenchyme) establishes the corticomedullary axis by which nephron distributions are patterned. Further elongation of the newly formed collecting duct network after birth is partly responsible for the postnatal growth of the kidney.

Elongation of the collecting ducts is regulated by oriented cell division, a process dependent on Wnt7b and Wnt9b.[190-192] Oriented cell division is characterized by the parallel alignment of the mitotic spindle of proliferating ductal epithelia with the longitudinal axis of the duct.

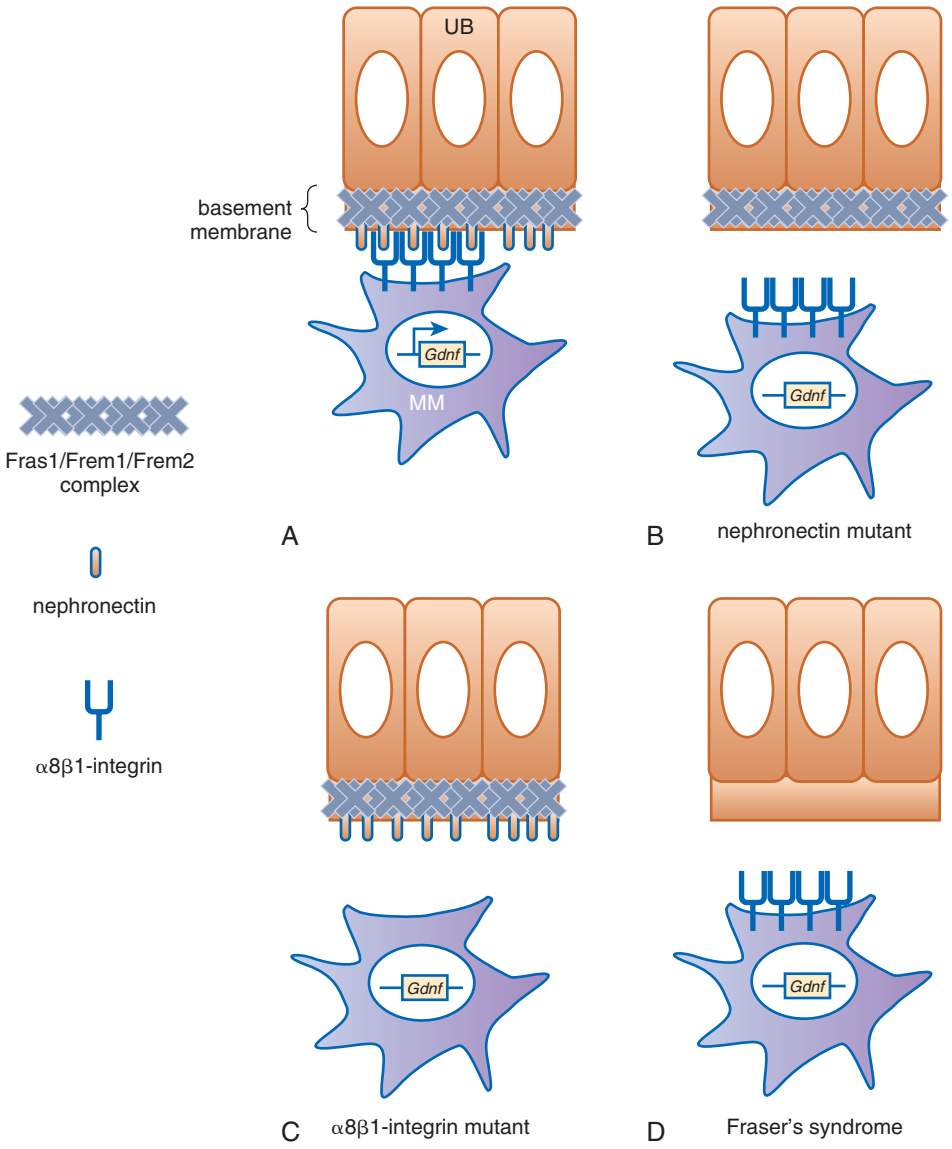

Figure 1.14 Molecular model of renal defect in Fraser's syndrome. A, Adhesion to the ureteric bud (UB) epithelium positively regulates the expression of glial cell–derived neurotrophic factor (GDNF) by the metanephric mesenchyme (MM). Adhesion and GDNF expression are impaired in the absence of **(B)** nephronectin (expressed by the UB), **(C)** α8β1 integrin (expressed by the MM), **(D)** or the Fras1/Frem1/Frem2 complex. Fras1, Frem1, and Frem2, which are implicated in Fraser's syndrome, are believed to coordinatedly anchor nephronectin to the UB basement membrane and stabilize the conjugation with α8β1 integrin. (Modified from Kiyosumi D, Takeichi M, Nakano I, et al: Basement membrane assembly of the integrin α8β1 ligand nephronectin requires Fraser syndrome-associated proteins. *J Cell Biol* 197:677-689, 2012.)

Oriented cytokinesis, therefore, guarantees that the daughter cells contribute to lengthening of the duct with minimal effect on tubular lumen diameter. The renal medulla and pelvis are nonexistent in mice lacking Wnt7b.[190] Notably, the collecting ducts and loops of Henle are stubbier, likely through disruption of oriented cell division. Wnt7b expression is restricted within the UB trunks and is absent in the ampullary UB tips. Oriented cell division of the collecting duct epithelia therefore requires reciprocal signaling with the surrounding interstitial stromal mesenchyme. Conditional inactivation of *Cttnb1 (β-catenin)* using a *Tcf21-Cre* transgene (which is expressed in the interstitial stroma) results in hypoplastic kidneys lacking medullary and papillary regions.[193] This is consistent with the possibility that the UB-stromal interaction via Wnt7b activates the canonical β-catenin–dependent Wnt signaling pathway. Wnt9b, another ligand expressed along the UB trunk region, has been identified as required for oriented cell division in collecting duct cells. In contrast, Wnt9b signals through a noncanonical Wnt pathway involving the activation of the small guanosine triphosphatase (GTPase) RhoA and the kinase Jnk.[191] Another mechanism that could contribute to elongation of the collecting ducts is convergent extension. Convergent extension involves the coordinated intercalation of elongated epithelial cells that thereby narrows and effectively lengthens the ducts. This mechanism was proposed on the basis of the reconfigured orientation of elongated cells in *Wnt9b* mutant collecting ducts.[191] How the interstitial stroma signals back to the UB to modulate oriented cell division and convergent-extension remains unknown.

The normal development of the collecting ducts also depends on cell survival cues provided by diverse ligands such as Wnt7b, EGF, and hepatocyte growth factor (HGF) and on interactions with the extracellular matrix.[190,194,195] Papillary collecting ducts display higher incidence of apoptosis in mice lacking Wnt7b or EGF receptor (EGFR).[190,194] Conversely, loss of Dkk1 (Dickkopf1), a secreted antagonist of Wnt7b, results in overgrowth of the renal papilla.[196] Conditional inactivation of *Dkk1* using the *Pax8-Cre* transgene (expressed in renal tubules and the collecting ducts) causes increased proliferation of papillary epithelial cells. The HGF receptor Met, $\alpha_3\beta_1$-integrin *(Itga3/Itgb1)*, and laminin α_5 *(Lama5)* are all required to maintain the expression of Wnt7b and thus are likely to support the viability of collecting duct cells.[134,195,197]

Poor development of the renal medulla and papilla are also observed in mutant mice lacking FGF7, FGF10, FGFR2, BMPR1A (ALK3), the components of the renin angiotensin aldosterone system (RAAS), Shh (Sonic hedgehog), or the orphan nuclear steroid hormone receptor Esrrg. FGF7 and FGF10 are the cognate ligands of FGFR2. Renal hypoplasia observed when *Fgfr2* is conditionally removed from the ureteric lineage is more severe than in mutants lacking Fgf7 or Fgf10, suggesting that these related ligands may have some functional redundancy in the development of the UB and collecting ducts.[69,198,199] Kidneys lacking *Bmp1ra* show an attenuated phosphorylation of SMAD1, an effector of the BMP and TGFβ ligands, and a concomitant increase in expression of c-Myc and β-catenin.[200] Although the significance of these results are not clear, the elevation of β-catenin indicates a novel crosstalk between BMP and Wnt signaling pathways in collecting ducts. Signaling through angiotensin is relevant to both early UB branching and the morphogenesis of medullary collecting ducts.[201] Genetic inactivation of angiotensinogen, its processing enzyme angiotensin-converting enzyme (ACE), and its target angiotensin-II AT1R receptors (Agtr1a and Agtr1b) results in similar phenotypes characterized by hypoplastic kidneys with modestly sized renal papillae.[202-207] Furthermore, the postnatal growth and survival of renal papilla grown ex vivo depend on the presence of AT1R.[208] Interestingly, in cultures of renal papilla explants, angiotensin appears to regulate the Wnt7b, FGF7, and $\alpha_3\beta_1$-integrin signaling pathways such that the loss of endogenous angiotensin or pharmacologic inhibition of AT1R causes significant dampening of the expression of *Wnt7b, Fgf7, Cttnb1,* and *Itga3/Itgb1.*[208] *Shh* is expressed in the more distal derivatives of the UB, the medullary collecting ducts and the ureter.[209] The germline deletion of *Shh* results in either bilateral renal agenesis or a single ectopic dysplastic kidney.[210,211] It has been shown that *Shh* controls the expression of early inductive and patterning genes (*Pax2* and *Sall1*), cell cycle regulators (N-myc and cyclin D1), and signaling effectors of the Hedgehog pathway (*Gli1* and *Gli2*). Interestingly, genetic removal of *Gli3* on an *Shh*-null background restores the expression of *Pax2, Sall1, cyclin D1, N-Myc, Gli1,* and *Gli2,* providing physiologic proof for the role of *Gli3* as a repressor of the Shh pathway in renal development.[211] Frameshift mutations resulting in truncation of the expressed Gli3 protein is linked to Pallister-Hall syndrome and the presence of hydronephrosis and hydroureter in both humans and mice.[212,213] Esrrg has a strong and localized expression within collecting duct epithelia later in gestation, and its inactivation in mice causes complete aplasia of the renal medulla and papillae. However, the ligand of Esrrg remains to be identified, and little is known about its downstream targets.

POSITIONING OF THE URETERIC BUD

A crucial aspect of kidney development that is of great relevance to renal and urologic congenital defects in humans relates to the positioning of the UB (see Figure 1.12*A*). Incorrect positioning or duplication of the bud leads to abnormally shaped kidneys and incorrect insertion of the ureter into the bladder, with resultant ureteral reflux that can predispose to infection and scarring of the kidneys and urologic tract.

Foxc1 (Forkhead box C1) is a transcription factor of the Forkhead family, expressed in the intermediate mesoderm and the MM adjacent to the wolffian duct. In the absence of *Foxc1*, the expression of GDNF adjacent to the wolffian duct is less restricted than in wild-type embryos. *Foxc1* deficiency results in ectopic UBs, hypoplastic kidneys, and duplicated ureters.[214] Additional molecules that regulate the location of UB outgrowth are Slit2 and Robo2, signaling molecules best known for their role in axon guidance in the developing nervous system. Slit2 is a secreted factor, and Robo2 is its cognate receptor. Slit2 is mainly expressed in the Wolffian duct, whereas Robo2 is expressed in the mesenchyme.[215] In one study, UBs formed ectopically in embryos deficient in either Slit2 or Robo2 similar to those in the *Foxc1* mutant. However, in contrast to the *Foxc1* phenotype, ureters in the *Slit2/Robo2* mutants undergo remodeling allowing their insertion into the bladder.[215] Instead, the ureters remained connected to the nephric duct in *Slit2* or *Robo2* mutants. The domain of GDNF expression is expanded anteriorly in the absence of either *Slit2* or *Robo2*. Indeed, mutations in *Robo2* have been identified in patients with vesicoureteral junction defects and vesicoureteral reflux.[216] The expressions of *Pax2, Eya1,* and *Foxc1,* all thought to regulate GDNF expression, were not dramatically different in the absence of *Slit2* or *Robo2,* suggesting that Slit/Robo signaling is not upstream of these genes. It is possible that Slit/Robo signaling is regulating the point of UB initiation by regulating the GDNF expression domain downstream of *Pax2* or *Eya1*. An alternative explanation is that Slit2 and Robo2 act independently of GDNF and that the expanded GDNF domain is a response to rather than a cause of ectopic UBs.

Spry1, as described earlier, negatively regulates the Ras/Erk signaling pathway and is expressed strongly in the posterior wolffian duct and the UB tips.[217] Embryos lacking *Spry1* develop supernumerary UBs, but unlike mutants of *Foxc1, Slit2,* or *Robo2,* they do not display changes in GDNF expression.[173] The phenotype of *Spry1* mutants can be rescued by reducing the GDNF expression dosage.[173] *Spry1* deletion also rescues the renal agenesis defect in mice lacking either Ret or GDNF.[172] Consistently, renal agenesis and severe renal hypoplasia, in mice expressing Ret specifically mutated on a tyrosine phosphorylation site known to couple with the Ras/ERK pathway, can be reversed in the absence of *Spry1*.[176] Thus, *Spry1* appears to regulate UB induction site by dampening RTK-dependent proliferative signaling.

Another negative regulator of branching is BMP4, which is expressed in the mesenchyme surrounding the wolffian duct. *Bmp4* heterozygous mutants have duplicated ureters, and in organ culture, BMP4 blocks the induction of ectopic UBs by GDNF-soaked beads.[218] Furthermore, knockout of gremlin, a secreted BMP inhibitor, causes renal agenesis, supporting a role for BMP in the suppression of UB formation.[219]

MOLECULAR ANALYSIS OF THE NEPHROGENIC ZONE

The continued replenishment of the reservoir of nephron progenitors during kidney development is crucial to guarantee generation of a sufficient number of nephrons. Fate mapping studies in mice using Cre driven by *Cited1* and *Six2* promoters demonstrate that the condensed mesenchyme, which aggregates around the UB, represents a pool of multipotent progenitors that replenishes itself and differentiates to give rise to all epithelial components of the nephron from podocytes to distal tubules.[18,19] Signaling through Wnt, FGF, and the BMP family of ligands is critical to maintain the delicate balance between progenitor self-renewal and differentiation toward a nephrogenic fate.

Wnt11 and Wnt9b, two ligands belonging to the Wnt family of signaling molecules, are expressed by the UB. The Wnt family was originally discovered as the wingless mutation in *Drosophila* and, in mammals, as genes found at retroviral integration sites in mammary tumors in mice. Wnt11 is highly expressed at the UB tips and decreased branching occurs in its absence, although it has no known specific effect on the induction of the epithelial transformation of the MM.[165] Wnt11 is a downstream target of Ret and is necessary to sustain GDNF expression in the MM.[76,144,145,165] Hence, Wnt11 participates in an autoregulatory feedback loop that maintains GDNF-Ret signaling to promote UB branching.[165] In contrast, Wnt9b, which is expressed in the entire UB except the very tips, appears to be the vital molecule expressed by the UB that induces the MM.[220] Wnt9b is not essential for the early induction of the UB or for the initial condensation of the MM. Further UB branching fails beyond the initial branching step resulting in T-shaped tubule (T-stage), however, likely because of downregulation of GDNF in the MM. The MM condenses up to the T-stage but the expressions of *Pax2, Eya1, WT1, Bmp7,* and *Six2* are distinctively diminished by 12.5 dpc in *Wnt9b* mutant mouse embryos. This loss of MM markers leads to failed induction of renal vesicles and tubulogenesis. Thus, Wnt9b is the closest candidate identified to date, which is likely to be the crucial molecule produced by the bud that stimulates induction of the nephrons.

A third member of the Wnt family, Wnt4, is expressed in pretubular aggregates and is additionally required for the epithelial transformation of the MM.[220,221] In *Wnt4* mutant embryos, pretubular aggregates failed to epithelialize into the tubular precursor of the mature nephron.[221] *Wnt9b*-deficient MM could be sufficiently induced in vitro to undergo tubulogenesis when grown with Wnt4-expressing fibroblasts.[220] In contrast, another study using the same co-culture assay showed that Wnt9b could not compensate for the loss of Wnt4. These findings suggest that Wnt9b and Wnt4 likely bind distinctive receptor complexes, with Wnt4 acting downstream of Wnt9b. Thus, a model has been proposed whereby Wnt9b acts as a paracrine factor, priming the MM to develop into renal vesicles expressing Wnt4. Wnt4 in this model functions as an autocrine factor required for commitment to a tubulogenesis program (Figure 1.15).

Two major Wnt signaling branches exist downstream of the Frizzled receptor (Fz): a canonical β-catenin–dependent pathway and a noncanonical β-catenin–independent pathway.[222] In the canonical pathway, Wnt-mediated signaling suppresses a phosphorylation-triggered pathway of proteosomal degradation, enabling the stabilization of β-catenin, which results in the formation of a complex between β-catenin and TCF/LEF (T-cell factor/lymphoid-enhancing factor) DNA-binding proteins that directly regulates transcriptional targets. Numerous studies demonstrate the importance of the canonical Wnt pathway for renal development: Conditional deletion of β-catenin from the cap mesenchyme completely blocks renal vesicle formation as well as expression of markers of induction such as Wnt4,

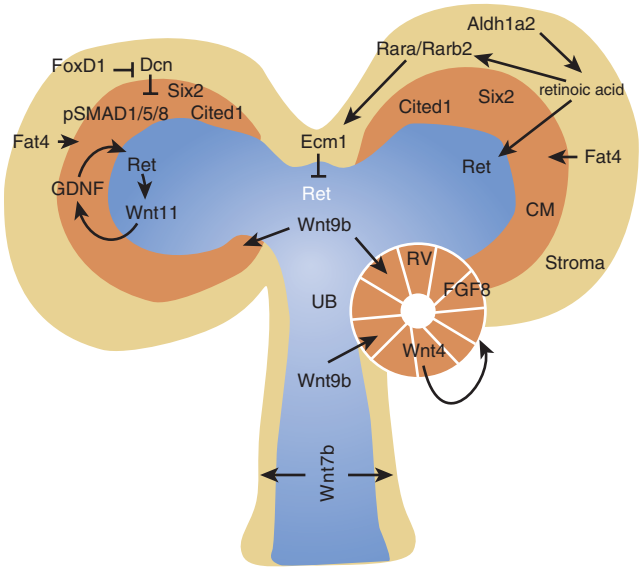

Figure 1.15 Tripartite inductive interactions regulating ureteric branching and nephrogenesis. Six2 and Cited1 are expressed in the self-renewing nephron progenitors within the cap mesenchyme (CM) surrounding the ureteric bud (UB). The UB tip domains express high levels of Ret, which is activated by glial cell–derived neurotrophic factor (GDNF) from the surrounding CM. Wnt11 is upregulated in response to Ret activation and stimulates GDNF synthesis in the CM. Wnt9b, expressed by the UB, and Fat4, expressed by the Foxd1-positive stroma, are required to initiate nephrogenesis from a subset of the CM. This results in the formation of a transient renal vesicle (RV) expressing FGF8 and Wnt4, factors that sustain epithelialization. The stroma expresses *Aldh1a2*, a gene required for retinoic acid synthesis, and genes for the retinoic acid receptors Rara and Rarb2. Retinoic acid signaling stimulates elevated expression of Ret in the UB tip domain while also suppressing Ret expression via Rara/Rarb2 and Ecm1 in the stroma to initiate bifurcation of the UB tip to generate new branches. Foxd1 in the cortical stroma also represses Dcn, thus relieving the Dcn-mediated suppression of BMP7-dependent signaling, which results in phosphorylation of SMAD1/5/8 (pSMAD1/5/8) and epithelialization of the cap mesenchyme. Wnt7b expressed in the UB stalk signals to the interstitial stroma and is an important factor that regulates cortico-medullary patterning of the kidney.

Fgf8, and Pax8.[223] By contrast, activation of stabilized β-catenin in the same cell population causes ectopic expression of mesenchymal induction markers in vitro and functionally rescues the defects observed in Wnt4- or Wnt9b-deficient mesenchymes. Inhibition of the kinase GSK3, a member of the β-catenin degradation complex, results in the ectopic differentiation of the MM.[224]

BMP7 is expressed in the UB and in the condensed MM.[225,226] Loss of BMP7 causes untimely depletion of the cap mesenchyme and nephrogenesis arrest.[225,226] BMP7 is thought to be a survival and proliferative factor for the cap mesenchyme, on the basis of organ culture experiments and the increased incidence of apoptosis observed within the presumptive nephrogenic zone of *Bmp7*-null kidneys.[226-229] The proliferative effect of BMP7 on nephron progenitors has been shown to depend on specific activation of the kinase Jnk leading to phosphorylation and activation of Jun and Atf2.[230] However, the cell-survival promoting functions of BMP7 are unlikely specific since BMP4 can functionally substitute for loss of BMP7 (based on phenotypic rescue in "knocked-in" mutants where *Bmp4* cDNA was inserted next to the endogenous *Bmp7* promoter).[231] The exact role of BMP4 in nephrogenesis is not known, although it has been described as important specifically within the UB lineage.[218] The transcription factor Trps1, an atypical member of the GATA family of transcription factors implicated in trichorhinophalangeal (TRP) syndrome, has been identified as a novel target of BMP7.[232] Trps1 expression is absent in *Bmp7*-null kidneys. *Trps1*-null mutant kidneys are hypoplastic and distinctively lacking glomeruli and renal tubules. Renal vesicle formation is distinctively compromised in the absence of Trps1, with a concomitant depletion of the cap mesenchyme. In cultured MM cells, the increased expression of E-cadherin following BMP7 stimulation is inhibited upon RNA interference–mediated knockdown of Trps1. Altogether these studies suggest that BMP7 acting through Trps1 is important for epithelialization of the cap mesenchyme.

The more primitive progenitors within the condensed mesenchyme express high levels of Cited1 and proliferate in a BMP7-dependent manner.[233] In response to BMP7, these Cited1-positive cells begin expressing Six2 and acquire responsiveness to Wnt9b. The exact role of Cited1 in the condensing mesenchyme remains poorly understood because *Cited1*- and compound *Cited1/Cited2*-knockout kidneys have apparently intact mesenchyme-to-epithelial transitions. It is not clear, however, whether the closely related Cited4 is upregulated and functionally compensates in the absence of Cited1 and Cited2.[234] Genetic inactivation of *Six2* causes premature and ectopic nephrogenesis.[19,235] The precocious epithelialization combined with increased incidence of apoptosis in *Six2*-deficient cap mesenchyme rapidly depletes the pool of nephrogenic precursors. The defective maintenance of nephrogenic precursors impairs reciprocal inductive interactions between the cap mesenchyme and the UB, causing overall stunting of kidney growth. Overexpression of Six2, on the other hand, prevented epithelial differentiation of the cap mesenchyme. Six2, therefore, is required to maintain the undifferentiated, self-renewing progenitor states of nephron precursors. Nevertheless, epithelialization in *Six2*-null mutants remains dependent on Wnt9b induction.[19] In the absence of Wnt signaling, Six2 constitutively represses expression of renal vesicle markers within nephron progenitors.[223] In response to Wnt induction, Six2 forms a complex with β-catenin and Lef/Tcf factors that regulate the expression of multiple genes required to coordinate mesenchyme-to-epithelial transition, including the upregulation of *Pax8, Fgf8, Wnt4,* and *Lhx1* and the attenuation of *Six2* expression. A fine-tuned activity of Six2 is therefore required to balance the maintenance of a pool of self-renewing nephron progenitors and to prime these progenitors for commitment to an epithelial fate via a canonical Wnt-dependent pathway.

The multidomain scaffolding proteins Dlg1 and CASK, members of the MAGUK (membrane-associated guanylate kinase) family of proteins, have been shown to be important in maintenance of nephron progenitor cells.[236] Dlg1 and CASK prominently localize at the plasma membranes of polarized cells, where they coordinate cell junction formation and assembly of protein complexes that regulate cell polarity.[237] In neurons, they are known to be important for organization of synapses.[238] The global deletion of *Dlg1* and *Cask* in mice led to severe renal hypoplasia and dysplasia with notable loss of the nephrogenic zone.[236] This renal phenotype was fully recapitulated when *Dlg1* and *Cask* were removed conditionally using either Pax3-Cre or Six2-Cre, suggesting that the defects are inherent within the MM compartment, particularly the nephrogenic precursors. Although UB branching was also decreased in the global and MM double-knockout mice, this defect proved to be secondary to depletion of the nephrogenic zone, because targeted ablation of *Dlg1* and *Cask* in the ureteric lineage using HoxB7-Cre did not cause renal hypoplasia or abnormal renal histology. Significantly diminished cell proliferation and increased apoptosis were observed in the nephrogenic zone in the absence of *Dlg1* and *Cask*. Consistent with the loss of the nephrogenic zone is the decreased expression of BMP7, Cited1, Six2, and FGF8. GDNF expression is also notably decreased, a finding that could explain the secondary impairment in ureteric branching. The concomitant reduction in BMP7 and FGF8 levels correlates with the dampening of signaling events downstream of Ras, including Erk, Jnk, and p38 MAPK pathways, possibly accounting for the loss of cell proliferation in the nephrogenic zone of *Dlg1/Cask* double-knockout mice.

The extracellular cues regulating Dlg1 and CASK functions in the nephrogenic mesenchyme are not yet clear. One possibility invoked is the interaction between Dlg1 and CASK with the FGF pathway via syndecan-2.[237] FGF2 is known to mediate condensation of the MM, whereas FGF8 is important for transition to Wnt4-expressing pretubular aggregates and renal vesicles.[215,239] FGF9 and FGF20, on the other hand, are important to maintain the stemness of nephron progenitors.[230] The corresponding receptors, FGFR1 and FGFR2, are crucial for the survival of the MM without which renal agenesis ensues.[240] Dlg1 and CASK are also likely to mitigate the proper migration and condensation of the nephron precursors around the UB. In compound heterozygous/homozygous *Dlg1/Cask* knockout subjects, kidneys were only modestly hypoplastic but showed a distinctively loose aggregation of Six2-expressing condensing mesenchyme.[236] This result is consistent with those of other studies showing that Dlg1 is important for directed cell migration of Schwann cells.[241,242]

MOLECULAR BIOLOGY OF NEPHRON DEVELOPMENT: TUBULOGENESIS

Gene targeting and other analyses have identified many genes involved in the initial induction of the metanephric kidney and the formation of the pretubular aggregate, but much less is currently known about how the pretubular aggregate develops into a mature nephron, a process through which a simple tubule elongates, convolutes, and differentiates into multiple distinct segments with different functions. Discussions of how this segmentation occurs have considered whether similarities will be found to other aspects of development, such as the limb or neural tube, where there is segmentation along various axes.

The Notch group of signaling molecules has been implicated in directing segmentation of the nephron. Notch family members are transmembrane proteins, the cytoplasmic domains of which are cleaved by the γ-secretase enzyme upon the interaction of the extracellular domain with transmembrane ligand proteins of the Delta and Jagged families, found on adjacent cells.[243] Thus, Notch signaling occurs between adjacent cells, in contrast to signaling by secreted growth factors, which may occur at a distance from the growth factor–expressing cells. The cleaved portion of the Notch cytoplasmic domain translocates to the nucleus, where it has a role in directing gene expression. Mice homozygous for a hypomorphic allele of *Notch2* have abnormal glomeruli, with a failure to form a mature capillary tuft.[244,245] Because null mutants of Notch family members usually result in early embryonic death, further analysis of Notch family function in kidney development has made use of the organ culture model.

When metanephric rudiments were cultured in the presence of a γ-secretase inhibitor,[31,246] expression of podocyte and proximal tubule markers was diminished in comparison with expression of distal tubule markers and branching of the UB. When the γ-secretase inhibitor was removed, there seemed to be a better recovery of expression of proximal tubule markers than of podocyte differentiation markers. Similar results were observed in mice carrying targeted mutation of the *Psen1* and *Psen2* genes that encode a component of the γ-secretase complex.[247] Conditional deletion of *Notch2* in the MM resulted in hypoplastic kidneys that did not develop glomeruli and proximal tubules, despite the presence of distal tubules and collecting ducts. Interestingly, the condensed mesenchyme and pretubular aggregates initiated epithelialization expressing Pax2 and E-cadherin but did not proceed to form S-shaped bodies. By contrast, Notch1-deficient metanephroi are phenotypically wild type, suggesting that Notch1 is not critical for cell fate determination during early nephron formation. Taken together, these studies seem to indicate that local activation of Notch2 during tubule morphogenesis is critical to determining the proximal cell fate after the epithelialization of renal vesicle.[248] The transcription factor Rbpj, the homolog of the *Drosophila* gene Suppressor of Hairless, is a transducer of canonical Notch signaling. Genetic inactivation of *Rbpj* in the MM leads to pronounced renal hypoplasia characterized by significant paucity in nephrons and the development of tubular cysts.[248,249] Fate mapping analyses reveal that Rbpj-deficient nephrogenic precursors develop into podocytes and distal tubules but not proximal tubules.[249] These findings further reiterate the crucial importance of canonical Notch signaling via Rbpj in the specification of the proximal segment of nephrons and the likelihood that Notch signaling independent of Rbpj arbitrates the determination of podocyte fate. Consistently, overexpression of the constitutively active Notch1 intracellular domain (N1ICD) drives the acquisition of proximal tubule fate in nephron precursors but inhibits the development of podocytes.[248]

The specification of the distal nephron fate requires the POU domain–containing transcription factor Pou3f3 (Brn1) and the metalloprotease genes *Adamts1* and *Adamts4*.[250,251] The proneural basic helix-loop-helix (bHLH) factor Ascl1 (MASH1) binds cooperatively with Pou3f3 and the related Pou3f2 (Brn2) to the promoter of the Notch ligand Delta1 to synergistically activate the transcription of Delta1 and stimulate neurogenesis.[252] Whether Pou3f3 is involved in regulation of Notch signaling in renal development is not clear. Germline deletion of *Pou3f3* results in defective patterning of the distal nephron segments.[251] Pou3f3 expression is first detectable in renal vesicles and becomes localized to the distal aspects of the comma- and S-shaped bodies, regions destined to become the distal convoluted tubules, the macula densa, and the loop of Henle. Without Pou3f3, elongation of prospective loop of Henle and overall maturation of distal nephron segments are arrested. Although the development of glomeruli, proximal tubules, and collecting ducts is seemingly not affected by the absence of Pou3f3, the severity of the distal nephron abnormalities causes renal insufficiency and perinatal death. The products of *Adamts1* and *Adamts4* are secreted thrombospondin domain–containing metalloproteases known to cleave a class of proteoglycans called *lecticans*. Null mutation of *Adamts1* in mice leads to hydronephrosis and is characterized by the thinning of the renal medulla and a distinctive paucity in the loops of Henle.[250,253] Lack of *Adamts4* appears benign but can exacerbate the simplification of the renal medulla due to loss of *Adamts1*.[254] As a consequence, mice with a compound null mutation of *Adamts1* and *Adamts4* mostly perish perinatally. This finding suggests that *Adamts1* and *Adamts4* have overlapping importance in the development of the distal nephron segment by a mechanism yet to be identified.

There is one example so far of a transcription factor involved in the differentiation of a specific cell type in the kidney. The phenotype is actually found in the collecting ducts, rather than in the nephron itself, but is discussed in this section because it is demonstrative of the kinds of phenotypes expected to be found as additional mutant mice are examined. Two cell types are normally found in the collecting ducts—principal cells, which mediate water and salt reabsorption, and intercalated cells, which mediate acid-base transport. In the absence of the Foxi1 transcription factor, only one cell type is present in collecting ducts, and many acid-base transport proteins normally expressed by intercalated cells are absent.[255]

In addition to cell differentiation, spatial orientation of cells is essential for tubule elongation and morphogenesis. In epithelia, cells are uniformly organized along an apical-basal plane of polarity. However, in addition, cells in most tissues require positional information in the plane perpendicular to the apical-basal axis. This type of polarization, referred to as *planar cell polarity*, is critical for morphogenesis of metazoans.[256,257] A study using cell lineage analysis and

close examination of the mitotic axis of dividing cells has shown that lengthening of renal tubules is associated with mitotic orientation of cells along the tubule axis, demonstrating intrinsic planar cell polarity.[192] Dysregulation of oriented cell division can give rise to cysts as a result of abnormal widening of tubule diameters.[258] To date, molecules implicated in planar cell polarity and tubule elongation include HNF1β-PKHD axis, Fat4, and Wnt9b.[191,192,259-263]

MOLECULAR GENETICS OF THE STROMAL CELL LINEAGE

The maintenance of reiterative ureteric branching and concomitant nephron induction largely accounts for the growth and enlargement of embryonic kidneys. Genetic studies reveal that interstitial stroma provides additional inductive cues that regulate UB branching and nephrogenesis (see Figure 1.15). These studies also underscore the pivotal role played by the stroma in establishing the stereotypical radial patterning of the kidney. In embryonic kidneys, the stroma is organized into two distinct zones, an outer stromal region within the nephrogenic zone expressing the winged helix transcription factor Foxd1/BF-2, and a deeper region expressing the basic helix-loop-helix transcription factor Tcf21 (Pod1/capsulin/epicardin).[22,23,264,265] Without either Foxd1 or Tcf21, UB branching and nephrogenesis are notably impaired, resulting in a distinctive perturbation of the corticomedullary renal histoarchitecture.[22,23,264]

The most prominent features of the genetic loss of Foxd1 include the thickening of the renal capsule and the formation of large metanephric mesenchymal condensates.[22,266] The morphologically altered renal capsule in *Foxd1* mutant kidneys has notably lost expression of *Aldh1a2/Raldh2* and *Sfrp1* (a regulator of Wnt signaling) and is abnormally interspersed with endothelial cells and Bmp4-positive cells.[266] The identity of these Bmp4-expressing cells populating the renal capsule in Foxd1-deficient kidneys is unknown, although on the basis of lineage tracing for Foxd1-promoter expression, the cells are clearly distinct from the presumptive medullary stroma. Bmp4 is a known chemotactic agent for endothelial cells,[267] so it is very likely that the ectopic Bmp4-positive cells account for the presence of endothelial cells within the broadened renal capsule of *Foxd1* mutant kidneys. The accumulation of the cap mesenchyme is also likely contributed in part by ectopic Bmp4 signaling in the absence of Foxd1, because Bmp4 has been shown to antagonize epithelialization of the cap mesenchyme.[267] Transcriptome analysis shows that the gene *Dcn,* which encodes for the collagen-binding proteoglycan decorin, is a specific target that is repressed by Foxd1 in the cortical interstitium.[268] *Dcn* expression is normally localized within the medullary stroma but is normally absent in the cortical stroma of wild-type kidneys. In the absence of Foxd1, decorin becomes abundantly expressed in the presumptive cortical stromal region. Functional cell-culture–based assays and epithelialization assays of mesenchymal aggregates demonstrate that decorin inhibits Bmp7 signaling and mesenchyme-to-epithelial transformation. The antagonistic effect of decorin on epithelial differentiation is further enhanced in vitro when the mesenchymal aggregates are grown in collagen IV, thus recapitulating the persistence of the cap mesenchyme as seen in Foxd1 mutant kidneys, in which both decorin and collagen IV are upregulated in the cortical interstitium. These findings are corroborated by the partial rescue of the *Foxd1*-null phenotype through genetic inactivation of *Dcn*.

Tcf21 (also called Pod1) is expressed in the medullary stroma as well as in the condensing MM.[264,265] Tcf21 is also expressed in a number of differentiated renal cell types that derive from these mesenchymal cells and include developing and mature podocytes of the renal glomerulus, cortical and medullary peritubular interstitial cells, pericytes surrounding small renal vessels, and adventitial cells surrounding larger blood vessels (see Figure 1.6).[193] The defect in nephrogenesis observed in *Tcf21*-null mice is similar to the defect seen in *Foxd1*-knockout mice, consisting of disruption of branching morphogenesis with associated arrest and delay in nephrogenesis. Analysis of chimeric mice derived from *Tcf21* mutant embryonic stem cells and EGFP-expressing embryos demonstrated both cell-autonomous and non–cell-autonomous roles for Tcf21 in nephrogenesis.[269] Most strikingly, the glomerulogenesis defect was rescued by the presence of wild-type stromal cells (i.e., mutant cells will epithelialize and form nephrons normally as long as they are surrounded by wild-type stromal cells). In addition, there is a cell-autonomous requirement for Tcf21 in stromal mesenchymal cells to allow differentiation into interstitial and pericyte cell lineages of the cortex and medulla, because Tcf21-null ES cells were unable to contribute to these populations.

Although many of the defects in the *Tcf21* mutant kidneys phenocopy those seen in the *Foxd1* mutant kidneys, there are important differences. Kidneys from *Tcf21*-null mice have vascular anomalies and defective pericyte differentiation that were not reported in *Foxd1* mutant mice. These differences might result from the broader domain of Tcf21 expression, which includes the condensing mesenchyme, podocytes, and medullary stromal cells in addition to the stromal cells that surround the condensates. In contrast to Foxd1, Tcf21 is not highly expressed in the thin rim of stromal cells found immediately beneath the capsule, suggesting that Foxd1 and Tcf21 might mark early and late stromal cell lineages, respectively, with overlap in the stroma that surrounds the condensates.[23] However, definitive co-labeling studies to address this issue have not been performed. Both Tcf21 and Foxd1 are transcription factors so it is interesting to speculate that they might interact or regulate the expression of a common stromal "inducing factor."

Retinoids secreted by the renal stroma are also recognized as important for the maintenance of a high level of Ret receptor expression in the UB tip, promoting the proliferation of UB epithelial cells and the growth of the ureteric tree.[9,270-272] One study concluded that the defective UB branching seen in *Foxd1*-null mutants is most likely a direct consequence of the loss of cortical expression of *Aldh1a2,* a gene involved in retinol (vitamin A) synthesis.[266] A later study has shown that renal stroma immediately around the UB tips is also important in regulating the bifurcation of the tips and the creation of new UB branches.[273] Autocrine retinoid signaling in the stromal cells juxtaposed to the UB tips stimulates the expression of extracellular matrix 1 (Ecm1). Ecm1 is specifically expressed at the UB cleft, where it suppresses and restricts Ret expression domains within the UB tips. In the absence of Ecm1, Ret expression in the UB tips

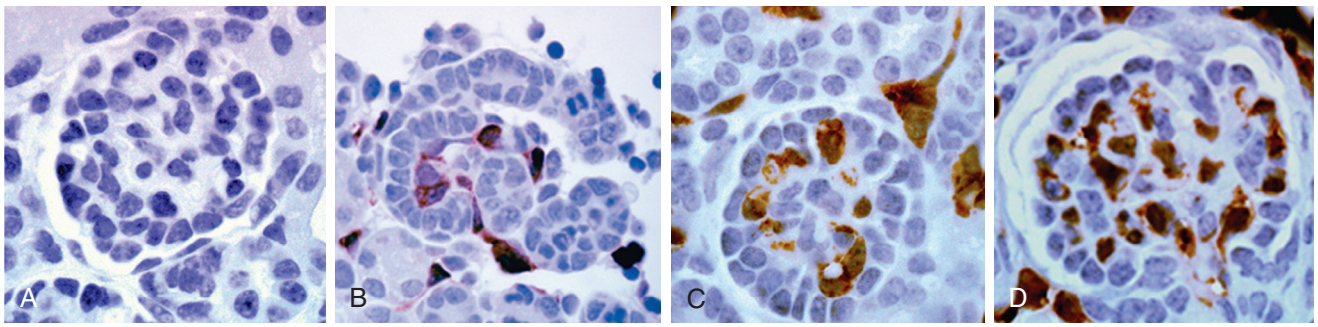

Figure 1.16 Developing glomeruli stained with an antibody to green fluorescent protein (GFP). Control glomerulus from a wild-type mouse. **A,** Comma-shaped body; **B,** S-shaped body; **C,** capillary loop stage; and **D,** mature glomeruli in the metanephros of an 18 dpc Flk1-GFP mouse strain. All endothelial cells express the GFP protein that is expressed under control of the endogenous Flk1/VEGFR2 promoter. (Reproduced with permission from the *Journal of American Society of Nephrology*.)

broadens, effectively attenuating UB branching through impaired formation of UB bifurcation clefts. Thus, stromal retinoids promote and confine Ret expression domains and, more likely, cell proliferation patterns within the UB tips.

A 2013 study has provided valuable insight into how stroma-based signaling intersects with UB-derived inductive cues to promote proper differentiation of the nephrogenic mesenchyme.[274] When the stromal lineage is selectively annihilated by Foxd1-Cre–driven expression of diphtheria toxin, the zone of condensing mesenchymal cells capping the UBs is abnormally broadened but the development of pretubular aggregates is strongly hindered. These findings reiterate those previously described in *Foxd1*-null mice, suggesting that regulation of nephrogenesis involves a crosstalk between stroma and UB-derived inductive signals. In particular, it was shown that Fat4-dependent Hippo signaling initiated by the stroma integrates with canonical Wnt signaling derived from the ureteric lineage in order to balance nephron precursor propagation and differentiation. The absence of Fat4 in the stromal compartment phenocopies the expansion of the nephrogenic precursor domain and failed epithelial differentiation of nephron progenitors seen in stroma-deficient kidneys. It was postulated that Fat4 acting through the Hippo pathway promotes the differentiation of the epithelial transition of nephrogenic precursors. This possibility was further reiterated by the rescue of the depletion of nephrogenic precursors by Fat4 deficiency in *Wnt9b*-knockout mice. Interestingly, the ablation of Vangl2, a signaling partner of Fat4 known to regulate renal tubular diameter,[259] fails to rescue the loss of nephron progenitors in *Wnt9b*-knockout animals, suggesting that Fat4-mediated signaling during early differentiation of nephrogenic precursors is independent of the planar cell polarity pathway.[274]

MOLECULAR GENETICS OF VASCULAR FORMATION

Vasculogenesis and angiogenesis both contribute to vascular development within the kidney. Endothelial cells may be identified through the expression of the tyrosine kinase receptor, VEGFR2 (Flk1/KDR).[275] Reporter mouse strains that carry β-galactosidase (lacZ) or GFP cDNA cassettes "knocked into" the *Vegfr2* locus permit precise snapshots of vessel development, because all the vascular progenitor and differentiated cells in these organs can be visualized either colorimetrically (with a β-galactosidase substrate) or by fluorescence (Figure 1.16). Use of other knock-in strains allows identification of endothelial cells lining arteriolar or venous vessels.[276]

Over the past decade, a number of growth factors and their receptors have been identified that are required for vasculogenesis and angiogenesis. Gene deletion studies in mice have shown that VEGF-A and its cognate receptor VEGFR2 are essential for vasculogenesis.[275,277] Mice that are null for the *Vegfa* gene die at 9.5 dpc from a failure of vasculogenesis, whereas mice lacking a single *Vegfa* allele (i.e., they are heterozygous for the *Vegfa* gene) die at 11.5 dpc, also from vascular defects.[277] These data demonstrate gene dosage sensitivity to VEGF-A during development. In the developing kidney, podocytes and renal tubular epithelial cells express VEGF-A and continue to express it constitutively in the adult kidney, whereas the cognate tyrosine kinase receptors for VEGF-A, VEGFR1 (Flt1), and VEGFR2 (Flk1/KDR) are predominantly expressed by all endothelial cells.[278] Which non-endothelial cells might also express the VEGF receptors in the kidney in vivo is still debated, although renal cell lines clearly do and MM cells express VEGFR2 in organ culture as outlined earlier.

Conditional gene targeting experiments and cell-selective deletion of *Vegfa* from podocytes demonstrated that VEGF-A signaling is required for formation and maintenance of the glomerular filtration barrier.[279,280] Glomerular endothelial cells express VEGFR2 as they migrate into the vascular cleft. Although a few endothelia migrated into the developing glomeruli of *Vegfa* podocyte conditional knockout mutants (likely because of a small amount of VEGF-A produced by presumptive podocytes at the S-shaped stage of glomerular development prior to Cre-mediated genetic deletion), the endothelia failed to develop fenestrations and rapidly disappeared, leaving capillary "ghosts" (Figure 1.17). Similar to the dosage sensitivity observed in the whole embryo, deletion of a single *Vegfa* allele from podocytes also led to glomerular endothelial defects known as endotheliosis that progressed to end-stage kidney failure at 3 months of age. As the dose of VEGF-A decreased, the associated endothelial phenotypes became more severe (Figure 1.18). Upregulation of the major angiogenic VEGF-A isoform (VEGFA^{164}) in developing podocytes of transgenic mice led to massive proteinuria and collapse of the glomerular tuft by 5 days of age. Taken together, these results show a requirement for

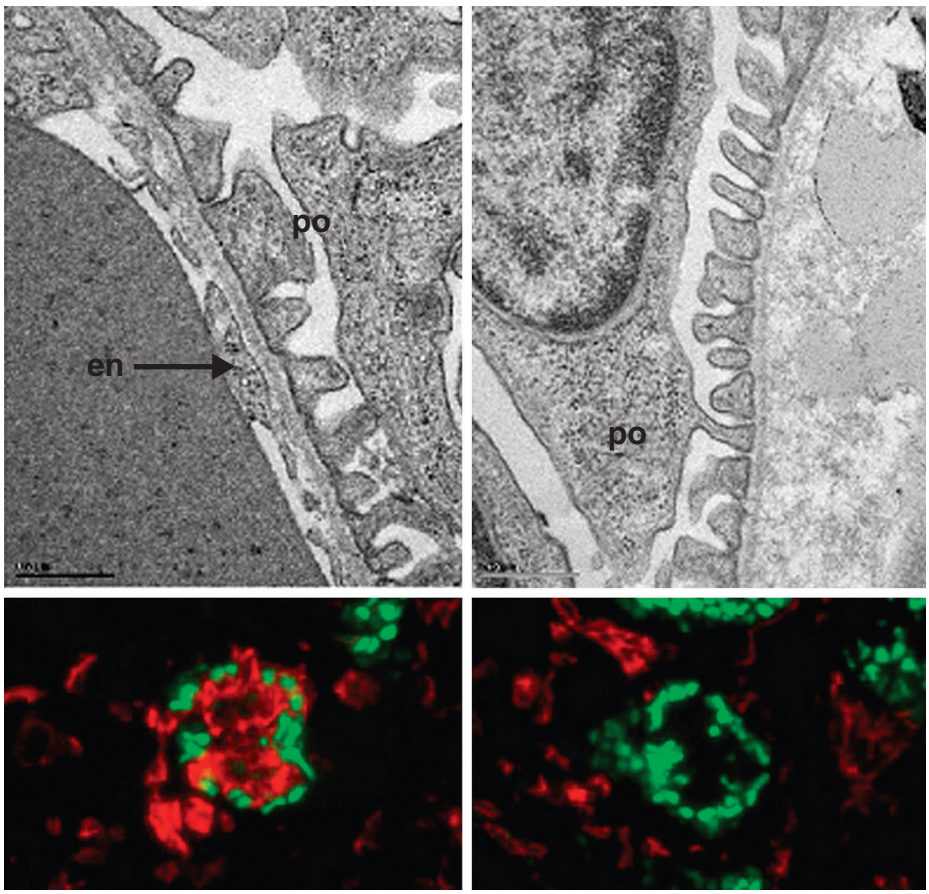

Figure 1.17 Top, Transmission electron micrographs of the glomerular filtration barriers from a wild-type mouse *(left)* and from a transgenic mouse with selective knockout of VEGF from the podocytes (*right*). Podocytes (po) are seen in both but the endothelial layer (en) is entirely missing from the knockout mouse, leaving a "capillary ghost." **Bottom,** Immunostaining of the barriers for WT1 (podocytes/*green*) and PECAM (endothelial cells/*red*) confirms the absence of capillary wall in VEGF knockouts. (Adapted from Eremina V, Sood M, Haigh J, et al: Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. *J Clin Invest* 111:707-716, 2003.)

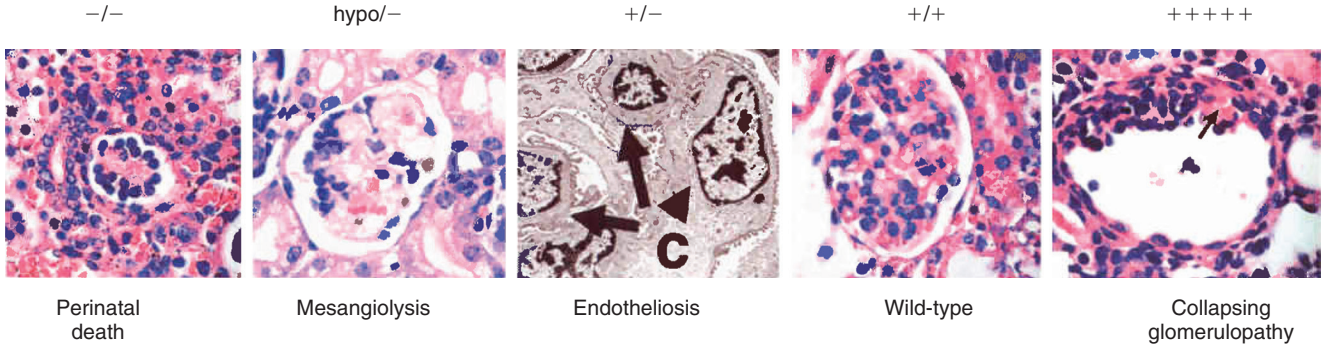

Figure 1.18 Effect of vascular endothelial growth factor dose on glomerular development. Photomicrographs of glomeruli from mice carrying different copy numbers of the VEGF gene within podocytes. A total knockout (loss of both alleles, –/–) results in failure of glomerular filtration barrier formation and perinatal death. A single hypomorphic allele (hypo/–) leads to massive mesangiolysis in the first weeks of life and death at 3 weeks of age. Loss of one copy (+/–) results in endotheliosis (swelling of the endothelium) and death at 12 weeks of age. Overexpression (20-fold increase in VEGF, +++++) results in collapsing glomerulopathy. (Adapted from Eremina V et al: Role of the VEGF-A signaling pathway in the glomerulus: evidence for crosstalk between components of the glomerular filtration barrier. *Nephron Physiol* 106:32-37, 2007.)

VEGF-A for development and maintenance of the specialized glomerular endothelia and demonstrate a major paracrine signaling function for VEGF-A in the glomerulus. Furthermore, tight regulation of the dose of VEGF-A is essential for proper formation of the glomerular capillary system. The molecular basis and mechanism of dosage sensitivity are unclear at present and are particularly intriguing, given the documented inducible regulation of VEGF-A by hypoxia-inducible factors (HIFs) at a transcriptional level. Nevertheless, it is clear that in vivo, a single *Vegfa* allele is