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ORIGINAL ARTICLE

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Renal branching morphogenesis: concepts, questions, and recent advances

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Abstract The ureteric bud (UB) is an outgrowth of the Wolffian duct, which undergoes a complex process of growth, branching, and remodeling, to eventually give rise to the entire urinary collecting system during kidney development. Understanding the mechanisms that control this process is a fascinating problem in basic developmental biology, and also has considerable medical significance. Over the past decade, there has been significant progress in our understanding of renal branching morphogenesis and its regulation, and this review focuses on several areas in which there have been recent advances. The first section deals with the normal process of UB branching morphogenesis, and methods that have been developed to better observe and describe it. The next section discusses a number of experimental methodologies, both established and novel, that make kidney development in the mouse a powerful and attractive experimental system. The third section discusses some of the cellular processes that are likely to underlie UB branching morphogenesis, as well as recent data on cell lineages within the growing UB. The fourth section summarizes our understanding of the roles of two groups of growth factors that appear to be particularly important for the regulation of UB outgrowth and branching: GDNF and FGFs, which stimulate this process via tyrosine kinase receptors, and members of the TGFβ family, including BMP4 and Activin A, which generally inhibit UB formation and branching.

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Introduction

The development of the metanephric kidney begins when the Wolffian (or nephric) duct, a simple epithelial tube derived from the intermediate mesoderm, gives rise to an outpocketing called the ureteric bud (UB). The UB then undergoes a complex process of growth, branching and remodeling, to eventually give rise to the entire urinary collecting system, including the collecting ducts, calyces, pelvis, and ureter. Unlike the situation in other organs that develop through epithelial branching morphogenesis, the tips of the UBs also induce some of the surrounding mesenchymal cells to convert to epithelia. These newly formed epithelia form the nephron, including the glomerulus, proximal tubule, loop of Henle, and the distal tubule, which is joined to a collecting duct by a connecting tubule (Saxen, 1987). The distal end of the ureter, which is at first connected to the Wolffian duct (WD), undergoes a process of remodeling and translocation resulting in its ultimate connection to the bladder (Batourina et al., 2002; Batourina et al., 2005).

Over the past decade, there has been significant progress in our understanding of renal branching morphogenesis and its regulation, and this review will consider several topics relevant to this process in which there have been recent advances. The first section deals with normal UB branching morphogenesis in rodents, and methods that have been used to describe it. The next section discusses a number of experimental methodologies that make mouse kidney development an attractive experimental system. The third section deals with the cellular processes that are likely to underlie UB branching morphogenesis (many of which are shared

with other developing epithelial organs), as well as cell lineages within the growing UB. The final section deals with a few of the growth factors that have been shown to play important roles in the regulation of UB outgrowth and branching. Many additional topics are not covered, and in these cases the reader is referred to other recent review articles.

Descriptive studies of renal branching morphogenesis *in vivo* and in culture

The evagination of the UB from the posterior end of the WD represents the initiation of renal branching morphogenesis and, as discussed below, some of the same signals that later control branching morphogenesis within the developing kidney are also involved in specifying the formation of a single UB at the correct position along the WD. In the mouse, UB formation is initiated at day E10.5. Once the primary UB has formed, it grows dorsally toward the metanephric blastema, a special population of mesenchymal cells also derived from the intermediate mesoderm. The blastema contains progenitors of the nephron (metanephric mesenchyme cells) as well as stromal progenitors, and this tissue has the specialized capability to induce the growth and branching of the UB in its characteristic pattern (Saxen, 1987). Once the tip of the UB has entered the metanephric blastema, it undergoes the first bifid branching event, yielding a T-shaped bud at E11.5 (Fig. 1A).

The early events of renal branching morphogenesis in the mouse have been studied both by serial reconstruction of sectioned kidneys at different stages, and by observing kidney development in organ cultures. When explanted at E11.5 and cultured on a filter at an air-medium interface, the kidney will grow for about 7 days; during this time, the early phases of UB branching morphogenesis and nephrogenesis occur in a manner closely approximating *in vivo* development (although at a lower rate). The visualization and analysis of UB branching has been greatly facilitated by the use of a transgenic strain that expresses eGFP (enhanced green fluorescent protein) specifically in the UB epithelium, under the Hoxb7 promoter (Srinivas et al., 1999a). Combined with the organ culture system, this allows time-lapse analysis of the pattern of UB branching (Fig. 1), and it also aids in the analysis of collecting system organization in kidneys that have been explanted at later developmental stages (Figs. 2A,2B).

Using the Hoxb7/eGFP mice, the early events in renal branching have been analyzed in some detail (Watanabe and Costantini, 2004; Caruana et al., 2006b). Time-lapse studies with these kidneys revealed that the UB is a highly plastic structure, which can branch in a variety of complex patterns, including terminal bifid

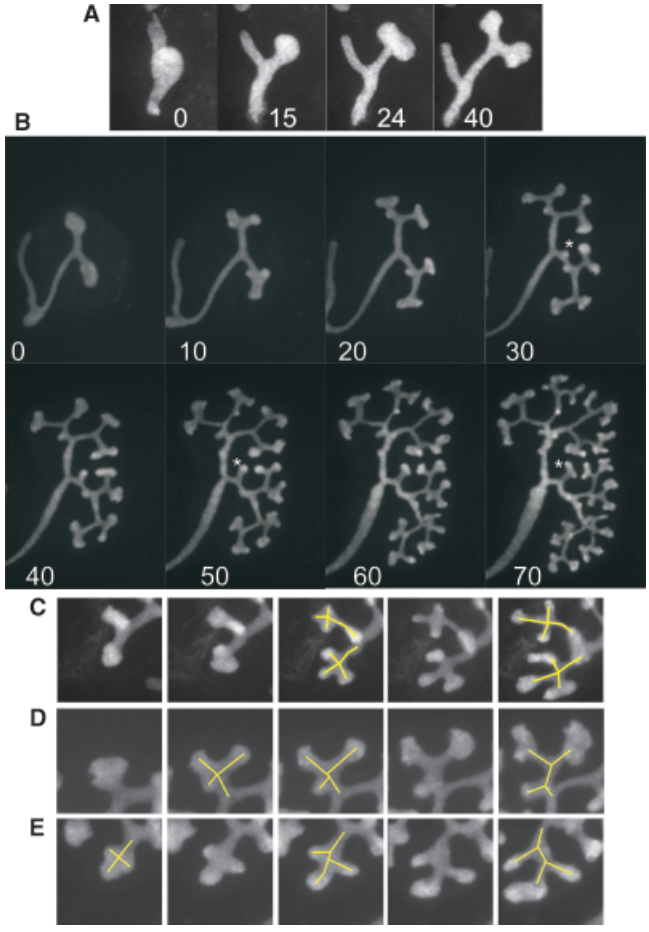

Fig. 1 Ureteric bud (UB) branching in organ culture. (**A**) The urogenital region from a Hoxb7/green fluorescent protein (GFP) transgenic embryo isolated at E10.5 and cultured for 40 hr, showing the outgrowth and initial branching of the UB (courtesy of Odyssé Michos and Rolf Zeller, University of Basel). (**B**) An E11.5 Hoxb7/GFP kidney (kidney "F") cultured and photographed at 10 hr intervals. The asterisks indicate a lateral branch that subsequently bifurcates. (**C**) Two examples of trifid branching, in which a single ampulla forms three new branches, which remain connected to the parental segment at a common branch point. (**D**) and (**E**), Examples of unequal trifid branching, in which the initial branch point is remodeled to produce two separate branch points. B–E are adapted from Watanabe and Costantini (2004) by permission of Elsevier.

(i.e., bifurcation), terminal trifid and lateral branching (Figs. 1B–1E). While the occurrence of lateral, bifid and trifid branching events had previously been inferred from static observations (Osathanondh and Potter, 1963a, 1963b, 1963c; Oliver, 1968; Al-Awqati and Goldberg, 1998; Majumdar et al., 2003) there is an inherent uncertainty in the origin of any branched structure that could only be overcome by examination of the same developing structure at successive times.

These studies confirmed the earlier inference, from static observations, that most UB branching events are terminal bifurcations. The tip of a UB branch first grows to form a swollen, rounded ampulla, which is then gradually remodeled into a laterally elongated

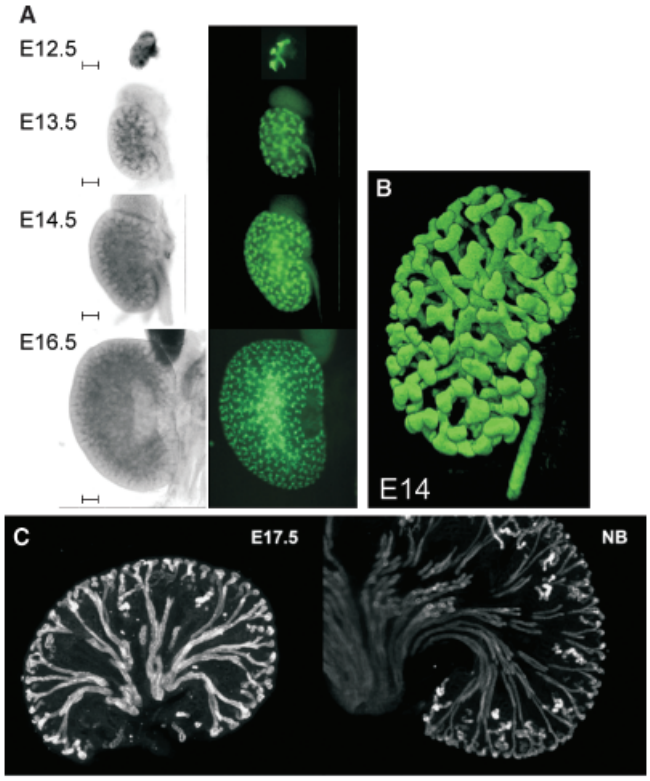

Fig. 2 Ureteric bud (UB) branching *in vivo*. (**A**) Expression of green fluorescent protein (GFP) in the UB of Hoxb7/GFP transgenic fetuses at E12.5 through E16.5. The images on the left are bright field photographs and those on the right were taken using standard fluorescein filters with ordinary wide field epifluorescence microscopy. (**B**) Optical sections of an E14 Hoxb7/GFP kidney were collected using a Bio-Rad laser scanning confocal microscope and volume rendered using Volocity software (Deborah Hyink, Mount Sinai School of Medicine, unpublished data). (**C**) Vibratome sections of E17.5 and newborn (NB) wild-type kidneys were stained with anti-calbindin D_{28K} to reveal the UB branches (Cebrian et al., 2004). Note the long, collecting duct segments with few branch points in the outer medulla, in contrast to the more extensive branching in the inner medulla and the outer cortex. A and C were reproduced from Srinivas et al. (1999a) and Cebrian et al. (2004) courtesy of the authors and John Wiley & Sons, Inc.

structure, and finally resolves into two new branches (recent data suggest that the UB trunk as well as the ampulla contributes to the next generation of branches—see below and Shakya et al., 2005b). In trifid branching, a single ampulla is remodeled into three rather than two new branches; trifid branching occurs frequently during the second round of branching (i.e., when the two tips of the E11.5 T-shaped bud undergo the next round of branching) and much less frequently during later branch generations (Majumdar et al., 2003; Watanabe and Costantini, 2004). While some trifid branch points are maintained during subsequent growth (Fig. 1C), others are later remodeled into two bifid branch points (Watanabe and Costantini, 2004) (Figs. 1D,1E). Thus "branch points" are not permanent structures, but can be transient. Another, more distinct type of branching is "lateral branching," the outgrowth of a new segment from the side of an existing segment, rather than from the terminal ampulla (Fig. 1B, asterisk); this accounts for only 6% of branching events in cultured mouse kidneys, and occurs mostly in the earliest generations of branches (Watanabe and Costantini, 2004). These time lapse studies showed that lateral branching did not alternate in a regular manner with terminal bifid branching, as is thought to occur during early UB branching in the human kidney (Al-Awqati and Goldberg, 1998). Whether this is a species difference, or simply reflects the difficulty of inferring branching patterns from fixed human specimens, remains unclear. In general, the subsequent occurrence of different types of terminal branching events could not be predicted from the developmental history of a particular segment. Thus, while the specific branching pattern of each kidney was not random, neither was it entirely predictable (Watanabe and Costantini, 2004). There is nothing known about the mechanisms that determine which mode of UB branching will occur at a particular time or location, and this remains a fascinating problem.

While time lapse analysis of kidney cultures also allows quantitative measurements of the rates of UB branching and elongation, these processes are somewhat retarded during organ culture, so their absolute values are not particularly relevant to the *in vivo* situation. However, they are useful for side-by-side comparisons of normal and mutant kidneys, or kidneys cultured under different experimental conditions. Another limitation is that only the earlier stages of organogenesis can be carried out in culture; a great many changes in the UB, and the collecting system that derives from it, take place at later stages, giving rise to the complex collecting system in the mature kidney, and contributing to the overall histoarchitecture of the kidney. Furthermore, collecting duct morphogenesis must be coordinated with the complex morphogenesis of nephron tubules and blood vessels (for a discussion of this topic, see Al-Awqati and Goldberg, 1998).

Some descriptive information about the later stages of UB branching morphogenesis has been obtained from studies of serially sectioned or dissected kidneys that developed *in vivo*. In one detailed study, UB branching was analyzed by serially sectioning mouse kidneys at stages from E11.5 to birth, staining with a UB-specific marker, and measuring the numbers of tips and branch points, the lengths of UB segments, and other parameters (Cebrian et al., 2004). This analysis suggested that the UB undergoes approximately eight generations of rapid and regular branching during the first few days of kidney development, and then on E15.5 enters a phase during which the inter-branch segments of the 6th–8th branch generation elongate extensively. This apparently gives rise to the long unbranched collecting ducts of the outer medulla (Figs. 2C,2D), which is required for urine concentration. The authors spec-

ulate that this extensive interstitial elongation is induced by medullary stromal cells, which are first evident around E15.5. This is followed by another two–three rounds of branching within the cortex, before birth (Figs. 2C,2D). This study also confirmed the belief that there is a positive relationship between the extent of UB branching and the nephron number (Al-Awqati and Goldberg, 1998; Clark and Bertram, 1999), but showed that the relationship is not linear throughout development: between E11.5 and E16.5, there are about 1,000 UB tips and 700 nephrons generated, while from E16.5 to birth, there is a 10-fold increase in nephrons but only a 2.7-fold increase in UB tips (Cebrian et al., 2004). Therefore, at the later stages many of the UB tips must induce multiple nephrons.

Branching morphogenesis during human kidney development has been studied primarily by micro-dissection of fixed fetal kidneys, and it is clearly more complex than that in the mouse. As in the mouse, there is thought to be an initial phase of extensive branching (15 generations), after which the 10th–15th generation of branches elongate dramatically, giving rise to the central medulla. At later stages, some of the UB tips induce several nephrons simultaneously, all of which are connected to a single collecting duct, or to the connecting tubule of another nephron, in arrangements known as "arcades," while others give rise to multiple lateral branches, each connected to a single nephron (Osathanondh and Potter, 1963c; Oliver, 1968; Ekblom, 1992; Al-Awqati and Goldberg, 1998). Arcades also occur in rodents, although they contain fewer nephrons (Saxen, 1987; Ekblom, 1992).

Understanding the mechanisms that control UB branching morphogenesis, determining when and where a growing epithelial tube will elongate, swell, narrow or branch, and in which direction the new branches will grow, is a fascinating and basic developmental problem that has attracted much interest in recent years. Furthermore, it has considerable medical significance. Failure to form the ureter (resulting in renal agenesis), as well as malpositioning or duplication of the ureter, are common birth defects, while more subtle defects in UB growth and branching may result in reduced nephron number, which can lead to renal disease later in life (Al-Awqati and Goldberg, 1998; Cullen-McEwen et al., 2001; Pohl et al., 2002; Poladia et al., 2006).

Experimental approaches to understanding renal branching morphogenesis

Several experimental systems that facilitate the study of renal branching morphogenesis have been developed. Renal cell lines, such as MDCK cells, have been used extensively for studies of epithelial morphogenesis, including branching. When MDCK cells are allowed to form cysts, the cysts can be induced to branch (Rosario and Birchmeier, 2003). While such cell lines have many advantages for studies of the basic molecular and cell biology of epithelial cells, their utility as a model of developmental processes has significant limitations. One important difference is that the mechanism of branching by MDCK cell cysts appears to be fundamentally different from UB branching during normal kidney development; the former involves an epithelial mesenchymal transition, followed by re-establishment of polarity and lumen formation, to generate new branches (Rosario and Birchmeier, 2003), whereas the latter involves the remodeling of continuous epithelial tubes (Meyer et al., 2004).

The analysis of kidney development in genetically modified mice has been instrumental in many of the advances in this field over the past decade. For gain-of-function experiments (i.e., examining the consequences of overexpressing or mis-expressing a protein), several promoter/enhancer sequences have been identified that permit expression of transgenes in specific cell lineages in the developing kidney. Two regulatory cassettes, from the Hoxb7 and Pax2 genes, have been used to direct expression to the entire WD and UB epithelium (Srinivas et al., 1999a, 1999b; Srinivas et al., 1999a; Kuschert et al., 2001; Chi et al., 2004). Several sequences that direct expression to different nephron segments or differentiated cell types (such as podocytes) have also been identified (for review, see Bianco et al., 2003; Gawlik and Quaggin, 2004). Regulatory elements that can direct transgene expression specifically in the renal stroma, undifferentiated metanephric mesenchyme, early nascent nephrons, or in specific sub-domains of the UB, have not yet been described. However, alternate methods exist to target the expression of any gene to these locations. These include: use of BAC transgenics (Bianco et al., 2003; Thivierge et al., 2006); targeting the gene of interest into a locus expressed in the desired location/stage of the developing kidney (Levinson et al., 2005; Oxburgh et al., 2005); and use of cell type-specific Cre recombinase transgenic lines (Gawlik and Quaggin, 2004; Igarashi, 2004) to activate the expression of a transgene in a specific cell lineage by deleting a transcriptional termination sequence (Soriano, 1999; Novak et al., 2000; Srinivas et al., 2001). In addition, temporal and tissue-specific regulation of transgenes in the WD and UB has been achieved through the use of the tet-On system of tetracycline-dependent regulation (Fig. 3) (Furth et al., 1994; Gossen et al., 1995; Urlinger et al., 2000; Shakya et al., 2005a). Finally, the Cre-Lox and Tet systems have been combined, by generating mouse strains in which the reverse tetracycline transactivator protein (rtTA) is turned on by Cre recombinase (Belteki et al., 2005; Yu et al., 2005); this will allow any of the cell type-specific Cre lines to be used to drive expression of rtTA, superimposing temporal and spatial control.

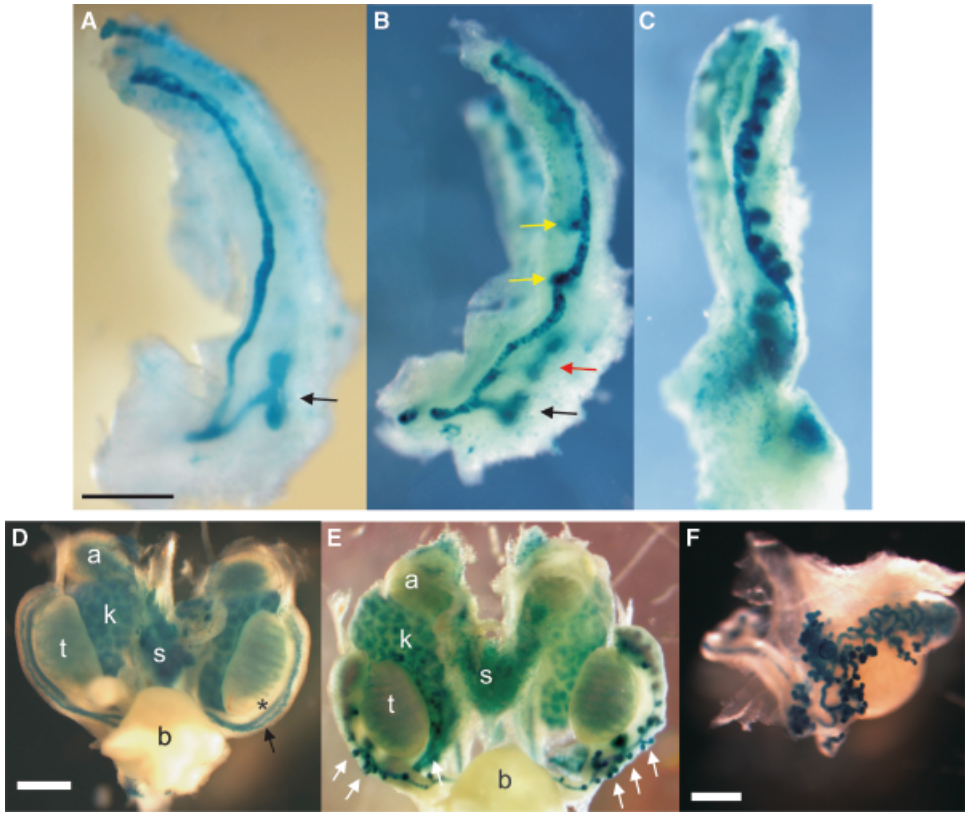

Fig. 3 Use of the Tet-on system to express a control lacZ transgene (**A**, **D**) or to misexpress GDNF in the Wolffian duct (WD) and ureteric bud (**B**, **C**, **E**, **F**). (A) A control Hoxb7/rtTA x TetO/lacZ bi-transgenic embryo with a single T-stage ureteric bud (UB) (arrow). (B) A Hoxb7/rtTA x BiTetO/lacZ/GDNF bi-transgenic embryo with a duplicated T-stage UB (red arrow) anterior to the normal UB (black arrow), and additional small ectopic buds along the WD (yellow arrows). (C) A Hoxb7/rtTA × BiTetO/lacZ/GDNF bi-transgenic embryo with multiple UBs along the WD. (D) Control Hoxb7/rtTA x TetO/lacZ bi-transgenic male, expressing lacZ in WD (asterisk) and Mullerian duct (arrow), as well as in UB branches in the kidney (k). a, adrenal; b, bladder; s, sympathetic ganglia; t, testis. E, F, Growth and branching of ectopic ureteric buds induced by GDNF at E14.5 (E) and E18.5 (F). E, Hoxb7/rtTA x BiTetO/lacZ/GDNF bi-transgenic male with multiple ectopic UBs (arrows). (F), highly branched ectopic UBs on the epididymis at E18.5. Scale bars, 0.5 mm. Adapted from Shakya et al. (2005a) by permission of Elsevier.

The analysis of knock-out mice has been an invaluable tool in the identification of genes that are required for normal UB branching (for reviews see Piscione and Rosenblum, 2002; Vainio and Lin, 2002; Yu et al., 2004) and a number of examples are discussed below. As useful as this approach has been, it has several inherent limitations. One is functional overlap or "redundancy" among multiple gene family members, which limits the phenotypic effects of many single gene knock-outs. This has been overcome in some cases by cross-breeding knock-out mutations in more than one gene (Patterson et al., 2001) or by the use of dominant-negative, transgene-encoded proteins to inhibit more than one gene product at a time (Celli et al., 1998). A second limitation is the early embryonic lethality caused by null mutations in some genes with later functions in the developing kidney (e.g., FGFR2 or BMP4, Winnier et al., 1995; Arman et al., 1998; Xu et al., 1998). This problem has been circumvented in some cases by the use of tissue-specific knock-out mice using various tissue-specific Cre transgenic lines expressed in the kidney (for recent reviews, see Gawlik and Quaggin, 2004; Igarashi, 2004). Another solution to the problem of embryonic lethality is the generation and analysis of mosaic mice in which the mutation of interest is only carried in some cells (which also carry a reporter gene, such as green fluorescent protein (GFP), to facilitate their identification), allowing the remaining wild-type cells to support development beyond the stage of lethality. As discussed below, this approach has been used recently to investigate the role of Ret signaling in the UB (Shakya et al., 2005b), and should be applicable to many other genes.

Another important tool for experimental studies of renal branching morphogenesis has been the ability to grow explanted kidney primordia in culture. This not only permits the direct observation of branching morphogenesis, using reporter strains such as Hoxb7/GFP (Watanabe and Costantini, 2004; Caruana et al., 2006b), but also allows a great variety of experimental manipulations. Much has been learned by simply culturing intact kidneys in culture media containing different growth factors, or drugs that inhibit specific

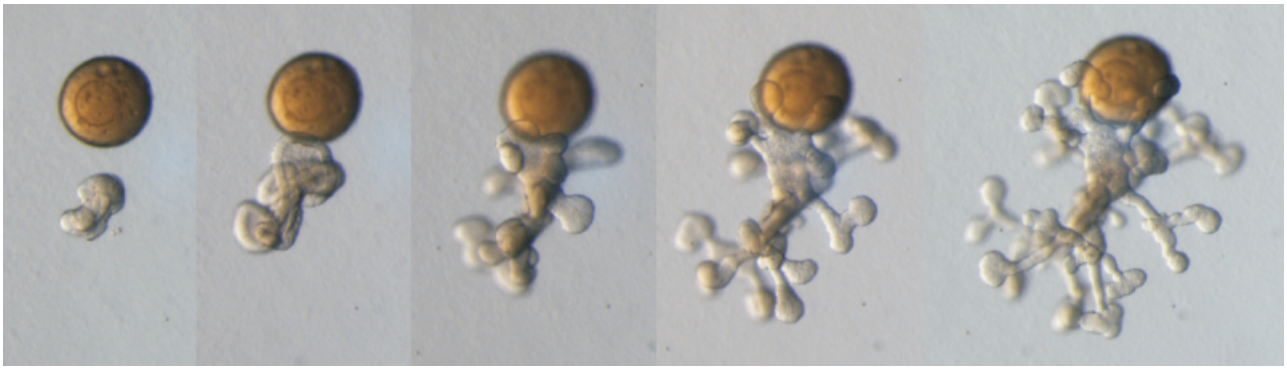

Fig. 4 Branching of the isolated ureteric bud (UB) in culture, using a modification of the method of Qiao et al. (1999a, 2001). The T-shaped UB from a wild-type E11.5 mouse kidney was separated from the mesenchyme/stroma and cultured in Matrigel, in medium containing 100 ng/ml GDNF, with an FGF10-soaked bead. The UB forms terminal ampullae that branch, and the trunks elongate, but the pattern of branching is not entirely normal (compare with Figs. 1 and 2). Photographs were taken at 24, 48, 72, 96, and 120 hr of culture (P. Riccio, B. Lu and F. Costantini, unpublished data).

signaling pathways or cellular functions. Furthermore, the UB can be physically separated from the mesenchyme, and "recombined" with mesenchyme from a kidney of different genetic composition, or from a different embryonic tissue (Grobstein, 1953a, 1955; Erickson, 1968). Such tissue recombination studies provided the earliest evidence that branching morphogenesis of the UB is controlled largely by signals from the metanephric mesenchyme, and that only metanephric mesenchyme has the capacity to support normal UB branching (Saxen, 1987). Fetal lung mesenchyme can also support UB branching but, interestingly, the pattern of branching is altered, displaying a higher frequency of lateral branching, which is a characteristic of lung branching (Lin et al., 2003). Thus, the mesenchyme surrounding the UB not only supports its growth but also appears to determine, at least in part, its pattern of branching.

A significant advance in the study of UB branching morphogenesis has come from the demonstration that the isolated UB can grow and branch extensively in the absence of any supporting mesenchyme, if placed in an artificial matrix with the right combination of conditioned media and/or purified growth factors (Perantoni et al., 1991; Qiao et al., 1999a) (Fig. 4). While the pattern of growth and branching of the isolated UB is not fully normal under these conditions (supporting the idea that the mesenchyme provides positional information), this nevertheless is a very useful system for identifying novel factors that influence UB branching (Sakurai et al., 2001), or for analyzing the effects of known growth factors on the UB (Qiao et al., 2001; Bush et al., 2004), free of the problem of indirect effects from mesenchymal or stromal cells.

Organ culture is often a powerful way to study the phenotypic defects caused by gene knock-outs or other germline genetic manipulations. However, while this can provide a relatively fast assay, the generation of knock-out and transgenic mouse strains is extremely time-consuming, and therefore has been a rate limiting step in manipulating gene expression during kidney development. Therefore, there is a need for methods to manipulate gene expression *directly* in cells of the cultured kidney, without having to generate new strains of mutant or transgenic mice. There has been limited, but encouraging, progress with several approaches to this problem. Viral vectors are able to infect cells in cultured kidneys (Herzlinger et al., 1992; Qiao et al., 1995), but until recently the UB has been inaccessible unless the mesenchyme is removed. This limitation has been overcome by the micro-injection of viral vectors directly into the UB lumen (Li et al., 2005; Polgar et al., 2005). The electroporation of naked DNA into kidney cultures has also been used to introduce genes into a sub-population of mesenchymal cells (Gao et al., 2005) but not yet into the UB. To inhibit gene expression, the use of small interfering RNAs (siRNA) appears to be extremely promising in many systems, and there have been a few reports of the use of this technique in the study of WD or UB branching *in vitro*. The expression of Activin A has been partially suppressed by lipofection of siRNA (small interfering RNA) into the rat WD (Maeshima et al., 2006), while reduced WT1 expression in the mesenchyme of cultured kidney Davies has been achieved by a similar method, causing defects in UB outgrowth or branching (Davies et al., 2004). Another new approach to the genetic manipulation of the kidney is to coax embryonic stem cells to contribute to the developing organ. Genetically marked ES cells injected into E12 or E13 kidney cultures have contributed to some epithelial tubules, although apparently not to the UB (Steenhard et al., 2005; Abrahamson and Steenhard, 2006). In other studies, teratomas generated by injecting ES cells into mice were found to contain tubules with the characteristics of WDs and UBs (Yamamoto et al., 2006); this suggests that it may be possible to coax ES

cells to contribute to UBs in organ cultures. While these technologies appear promising, much additional work is needed before they could replace germ line genetic manipulation.

In order to delineate the genetic control of renal branching morphogenesis, it will be essential to have a thorough description of normal patterns of gene expression in the developing kidney. There is a large and increasing collection of data on the patterns of expression of individual genes, often based on *in situ* hybridization (Davies, 1999), as well as on global gene expression profiles derived from microarray studies with whole kidneys at different stages (Stuart et al., 2001, 2003; Schwab et al., 2003), as well as micro-dissected tissues such as UBs and metanephric mesenchyme (Stuart et al., 2003; Schmidt-Ott et al., 2005; Caruana et al., 2006a). An NIH-supported consortium, GUDMAP: A Molecular Atlas of Genitourinary Development (www.gudmap.org), is currently collecting both types of data on a large scale and making it available to the scientific community.

Cellular processes driving renal branching morphogenesis

Cell lineage in the UB

Although some exchange of cells between the UB and the metanephric mesenchyme/nephron lineage has been observed in organ culture (Herzlinger et al., 1993; Qiao et al., 1995), *in vivo* the UB epithelium seems to be a discrete cell lineage, which grows without either incorporating cells from the mesenchyme or releasing cells that contribute to other structures. This has been shown most clearly by studies in which a transgenic mouse line expressing Cre recombinase in either the UB or MM was mated with a Cre-reporter strain, which turns on a reporter gene such as lacZ, and maintains its expression in all daughter cells (Soriano, 1999; Oxburgh et al., 2004; Kobayashi et al., 2005). The daughters of cells labeled by a UB-specific Cre gene were only found within the UB at later stages, while those labeled by a MM-specific Cre did not contribute to the UB. Therefore, the new cells required for the growth of the UB must arise exclusively through cell proliferation.

Within the UB, the location of proliferating cells has been examined mainly at early stages of kidney development, when very active branching is occurring, and at these stages the highest rates of proliferation are in cells close to the tips of the UB branches, with much lower numbers of proliferating cells in the trunks (Fisher et al., 2001; Meyer et al., 2004; Michael and Davies, 2004). These data are consistent with the rapid expansion of UB tips to form the ampullae, which give rise to the next generation of branches. This suggests that ''tip cells'' are likely to serve as the progenitors of trunk cells, since as branching proceeds, the ratio of tips cells to trunk cells decreases greatly; since the tip cells are rapidly proliferating, many of them must be recruited to generate the new trunks.

Such a model received direct support from studies in which genetically mosaic kidneys, expressing Hoxb7/GFP in only a small proportion of UB cells, were followed during growth in organ culture (Shakya et al., 2005b). When GFP+cells located in a UB tip at the start of the culture were traced over time, they initially remained at the tip and increased in number, while the adjacent trunk elongated without incorporating any labeled tip cells; however, after the tip had branched, some of the GFP+daughter cells remained in the new tips, while others had contributed to the new trunks (Fig. 5). This process did not involve retrograde cell migration from tip to trunk but, rather, some tip cells appeared to stay behind in the newly formed trunk as the tip grew forward. This observation suggested that tip cells are bipotential, and give rise to both trunk and tip cells (Shakya et al., 2005b). The possibility that trunk cells can give rise to tip cells has not been directly examined, but the phenomenon of lateral branching (in which a new tip forms from the trunk of an existing branch) implies that this transition can also occur.

The conclusion that UB tips are the major growth centers of the UB is based on observations of the early growth of the kidney in culture, at the stage during which rapid, repeated branching is occurring. During later kidney growth, when extensive elongation of the collecting ducts occurs (Oliver, 1968; Cebrian et al., 2004), it is likely that the ducts grow largely by an intrinsic mechanism (e.g., internal cell division, changes in cell shape, or convergent extension—see below) rather than by the acquisition of new cells from the tip.

The distinction between UB ''tip'' and ''trunk'' cells is not based exclusively on their location in the UB, but is also reflected in patterns of gene expression—many genes examined by *in situ* hybridization or micro-dissection are expressed preferentially or exclusively in the UB tips or in the trunks (Davies, 1999; Schmidt-Ott et al., 2005; Caruana et al., 2006a). Therefore, when a tip cell gives rise to a trunk cell it must significantly alter its pattern of gene expression. At least some of the differences in gene expression patterns between UB tip and trunk appear to be an intrinsic property of the growing UB, rather than being imposed by signals from the surrounding cells in the kidney. This conclusion is based on two observations: (1) in transgenic mice misexpressing GDNF in the WD, which form ectopic UBs from the WD in extra-renal regions, these ectopic buds express tip markers (Ret, Gfra1, Wnt11) only at their tips, even though they are in a ''foreign'' environment, surrounded by cells that appear distinct from normal renal mesenchyme or stroma (Shakya et al., 2005a); and (2) isolated UBs, cultured in the absence of mesenchymal cells, also continue to express tip markers only at their

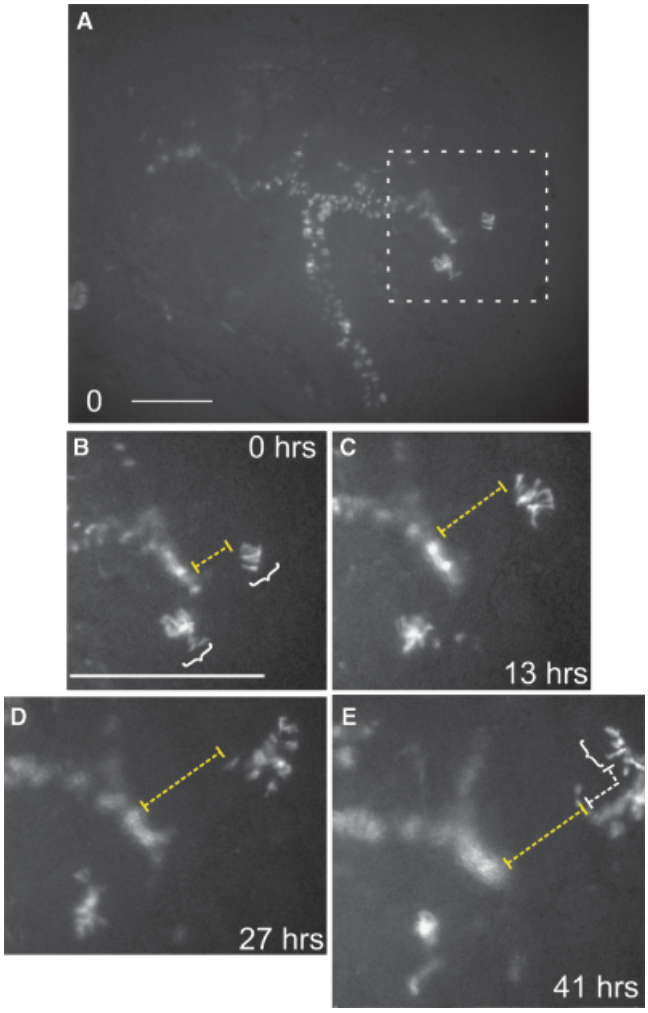

Fig. 5 Fate of tip cells during ureteric bud (UB) elongation and branching. (**A**) Cultured kidney from a chimeric mouse embryo generated by injecting ES cells carrying the Hoxb7/green fluorescent protein (GFP) transgene into a wild-type blastocyst. (**B**–**E**), Enlargements of the boxed area in A, at the indicated times of culture. B, Two clusters of GFP+cells are initially located at the tips (brackets), adjacent to a segment of UB trunk devoid of GFP+cells (yellow dashed line). During initial elongation (C), the GFP+cells remain in the tip, and increase in number, while the trunk elongates without incorporating cells from the tip. Starting at 27 hr (D–E), the GFP+cells became more widely distributed, some remaining at the tip (bracket in E) and others remaining behind in the new trunks (white dashed lines). Scale bars, 0.25 mm. Modified from Shakya et al. (2005b) by permission of Elsevier.

tips, not in their trunks (B. Lu and F. Costantini, unpublished data). How the tip and trunk maintain their distinct identities is an interesting problem.

Cellular mechanisms of branching

How does a straight epithelial tube give rise to a branched tube during UB morphogenesis? It is first important to point out that the continuity of the UB epithelium and its lumen remains intact during UB growth and branching (Meyer et al., 2004). Few if any cells delaminate from the epithelium and rejoin it later, nor are new branches formed by solid clusters of cells that later reform an epithelium. Instead, the bud epithelium gradually changes shape without losing its integrity or polarity. There are many cellular processes that, in principal, could drive such changes in epithelial shape, including intrinsic (active) mechanisms such as localized cell proliferation or cell death, cell migration, or changes in cell shape or cell adhesion; and extrinsic (passive) mechanisms such as forces exerted on the epithelium by the extracellular matrix or by surrounding cells or tissues (Ettensohn, 1985; Hogan, 1999; Pilot and Lecuit, 2005). It is not yet clear to what extent each of these mechanisms contributes to UB branching, but several of them have been implicated.

Localized cell proliferation appears to contribute to the evagination of the primary UB from the WD, as well as the formation of ampullae at the UB tips. Proliferation rates are higher on the side of the WD where the UB is beginning to form (Michael and Davies, 2004), and also much higher in UB tips than in trunks (Fisher et al., 2001; Meyer et al., 2004; Michael and Davies, 2004). Localized proliferation could drive the transformation of a rounded ampulla to a branched structure, if it occurred preferentially at two opposite ends of the ampulla and was reduced in the middle (i.e., in the forming cleft). While one study using isolated UBs noted a higher rate of proliferation in regions of the ampulla giving rise to new buds (Meyer et al., 2004), more extensive and quantitative studies are required to resolve this issue. In the case of lung epithelial branching, a specific attempt to detect such localized proliferation coupled to branching failed to provide evidence for such a mechanism (Nogawa et al., 1998). Localized apoptosis could also contribute to branching, by reducing cell number in the forming cleft while the cell number increases in the two forming branches. However, while one study (Meyer et al., 2004) revealed a higher number of apoptotic cells in trunks than in ampullae of the isolated UB growing in culture, there were not increased numbers of apoptotic cells in branch points. Furthermore, the overall number of apoptotic cells in normally growing UBs is extremely low (Coles et al., 1993), suggesting that cell death is not likely to be a major factor in UB branching morphogenesis.

UB branching could be driven by the directed migration of epithelial cells towards the two (or three) poles of the ampulla. Directed cell migration within the epithelium (in response to FGF) has been described as an important process driving the morphogenesis of the air sac in late *Drosophila* larvae (Cabernard and Affolter, 2005), and a similar mechanism might contribute to UB branching. There appears to be extensive cell motility within the UB epithelium, as shown by time lapse microscopy of cultured kidneys mosaic for the expression of the Hoxb7/eGFP gene (Shakya et al., 2005b).

However, it was not clear from this study whether the cell movements are directed or random. One source of support for a cell migration model is the observation that GDNF, a factor secreted by the MM, promotes both the branching of the UB and the directed migration (i.e., chemotaxis) of cultured cells expressing the GDNF receptor, Ret (Tang et al., 1998) (see further discussion of this model below). However, it remains to be determined if cell migration is indeed important for UB branching.

Yet another potential driving force for branching is a change in cell shape mediated by the cytoskeleton: the concerted transformation of epithelial cells from a cuboidal to a wedge shape could cause localized folding of the epithelial sheet, leading to branch initiation (Ettensohn, 1985). Meyer et al. (2004) observed that UB trunk cells are predominantly cuboidal, while wedge-shaped cells with a larger basal aspect were found in the ampullae and in forming outpouches from the ampullae (i.e., sites of new branch formation). Furthermore, they saw strong actin staining along the apical surface of the wedge-shaped cells, suggesting localized contraction of the actin/myosin cytoskeleton, as well as the localization of Ezrin, an actin-binding protein involved in cell motility, at the apical surface of cells in the ampullae. Based on these observations, they proposed that UB branching may be driven by a ''purse-string'' model (Ettensohn, 1985), in which contraction of an apical actin ring leads to formation of wedge shaped cells, resulting in a smooth out pocketing of the epithelium. Michael et al. (2005) showed that treatment of kidney cultures with drugs that disrupt actin micro-filaments, or block the tension-producing activity of myosin on actin, results in bloated, misshapen UBs with reduced branching, which is consistent with such a model.

Other UB-intrinsic mechanisms that might contribute to local branch initiation include local changes in cell adhesion, or the local remodeling of the basement membrane by matrix-degrading enzymes secreted by specific subsets of UB cells (e.g., matrix metalloproteases) (Al-Awqati and Goldberg, 1998; Meyer et al., 2004).

While it is possible that extrinsic physical forces exerted by the surrounding cells also contribute to UB branching, the ability of the isolated UB to branch extensively in an artificial matrix (Perantoni et al., 1991; Qiao et al., 1999a) indicates that this is not an essential process for branching. As the overall pattern of branching morphogenesis differs somewhat between the intact kidney and the isolated UB, cells outside the UB apparently modify and refine the pattern of growth and branching. However, this may be accomplished through the local activity of growth factors, matrix components or matrix-altering enzymes, or other factors secreted by the mesenchyme, rather than by physical forces.

In addition to the decisions of when, where, and at what angle to branch, the overall pattern of the developing collecting system is influenced by other processes, including elongation, widening and narrowing of the UB trunks, and more complex types of epithelial reshaping (such as the remodeling of a single trifid branch point into two bifid branch points, Watanabe and Costantini, 2004). Other than an obvious requirement for cell proliferation for the overall growth of the UB, the cellular mechanisms underlying these processes remain unknown. However, it is interesting to speculate that elongation of the UB trunks, which is often accompanied by narrowing, could be accomplished by convergent extension, a process of cell intercalation that leads to lengthening of groups of cells during other developmental events such as germ band extension in Drosophila and gastrulation in vertebrates (for a recent review, see Keller, 2006).

Signals and receptors controlling UB branching morphogenesis

In principle, the signals that promote and direct UB branching morphogenesis could be derived from a variety of sources, including the UB itself as well as any other cell population in the developing kidney, including the metanephric mesenchyme, nephron progenitors, stroma, or vascular cells. While evidence for a major role of the MM has existed for decades (Grobstein, 1953b, 1955; Erickson, 1968), recent data implicate several other cell types as well. The fact that the isolated UB can branch (Meyer et al., 2004) implies that localized, autocrine signals from the UB epithelium itself cause buds to form in some regions while inhibiting them in others. Mutations in genes expressed in cortical stromal progenitors can affect UB branching, implying that signals from these cells (yet to be identified) act on the UB (Hatini et al., 1996; Mendelsohn et al., 1999; Batourina et al., 2001; Levinson and Mendelsohn, 2003; Cullen-McEwen et al., 2005); in addition, BMP4 (bone morphogenetic protein 4) expressed in stromal cells surrounding the WD and primary UB appears to regulate UB outgrowth, as discussed below. Another recent study has provided evidence that a signal produced from angioblasts in the kidney primordium, in response to MM-derived Vegfa, is also needed to maintain early UB branching (Tufro-McReddie et al., 1997; Gao et al., 2005).

While several of the aforementioned signals remain hypothetical, a large number of specific growth factors have been implicated, with varying degrees of certainty, in the control of renal branching morphogenesis. This topic has been extensively reviewed in recent years (Davies, 2002; Piscione and Rosenblum, 2002; Vainio and Lin, 2002; Carroll and McMahon, 2003; Vainio, 2003; Shah et al., 2004) and this paper will not include a complete survey. Instead, it will focus on two general

classes of growth factors whose roles in UB branching have been the subject of recent advances, and which appear to serve generally opposing roles in the regulation of this process: GDNF and FGFs (fibroblast growth factors), which signal through tyrosine kinase receptors, and promote UB growth and branching; and several members of the TGFβ (transforming growth factor β) family, which appear to provide mainly negative regulation.

GDNF and FGFs

GDNF: GDNF is the growth factor most extensively implicated in UB branching morphogenesis (reviewed in Sariola and Sainio, 1997; Costantini and Shakya, 2006). Although a distant member of the TGFβ superfamily, GDNF, unlike other members of this family, signals primarily through a receptor tyrosine kinase, Ret, together with the co-receptor Gfrα1 (Takahashi, 2001; Arighi et al., 2005). GDNF is first expressed in the metanephric mesenchyme adjacent to the caudal region of the WD, from which the UB will emerge, while Ret and Gfrα1 are expressed throughout the WD epithelium (Pachnis et al., 1993; Hellmich et al., 1996; Baloh et al., 1997; Sainio et al., 1997). Knock-out of any one of these genes results most frequently in renal agenesis, due to failure of the UB to emerge from the WD (Schuchardt et al., 1994; Moore et al., 1996; Pichel et al., 1996; Sanchez et al., 1996; Cacalano et al., 1998; Enomoto et al., 1998). This, together with the ability of ectopically expressed or applied GDNF to induce the formation of supernumerary UBs from the WD, in explants of E10.5–E11.5 urogenital regions (Vega et al., 1996; Sainio et al., 1997) or *in vivo* (Shakya et al., 2005a) (Fig. 3), revealed that one of the first roles of GDNF in the mammalian excretory system is to promote UB outgrowth. However, GDNF is apparently not the only signal that contributes to this event, because even in the absence of GDNF, Ret or GFRα1, the UB forms in approximately the correct location in a fraction ($\sim$ 20%–40%) of mutant embryos (Schuchardt et al., 1996). Further evidence for the importance of GDNF has come from studies of several other genes (including transcription factors as well as other signaling molecules or receptors), which have been found to regulate, either positively or negatively, the level, timing or spatial extent of GDNF expression. Mutations in these genes result in either the failure of UB outgrowth, or the formation of ectopic, supernumerary UBs, as a consequence of altered GDNF expression (for recent reviews of this topic, see Bouchard, 2004; Costantini and Shakya, 2006).

As the UB extends into the metanephric blastema and begins to branch, Ret and GFRα1 become downregulated in the trunks of the UB and restricted to the tips, while GDNF expression becomes restricted to the peripheral, undifferentiated mesenchyme surrounding the UB tips, and this pattern persists throughout kidney development. GDNF signaling via Ret is important for continued UB branching morphogenesis, based on several types of evidence. These include the reduced UB growth and branching caused by murine mutations that reduce (but do not eliminate) GDNF/Ret signaling (Pichel et al., 1996; Batourina et al., 2001; de Graaff et al., 2001; Jijiwa et al., 2004), and studies in which GDNF or anti-GDNF antibodies were added to cultured kidneys (Vega et al., 1996; Pepicelli et al., 1997; Towers et al., 1998; Ehrenfels et al., 1999) or isolated UBs (Qiao et al., 1999a) (reviewed in Costantini and Shakya, 2006).

At least three of the well-characterized signaling pathways downstream of Ret, the Ras/Erk MAP kinase, PI3-kinase/Akt, and PLC-γ/calcium pathways, serve important roles in UB growth and/or branching. In organ culture, chemically inhibiting either PI3-kinase or PLC-γ severely inhibits UB growth and branching (Tang et al., 2002; Jain et al., 2006), while inhibiting the Erk pathway reduces the rate of branching while only slightly inhibiting elongation (Fisher et al., 2001; Watanabe and Costantini, 2004). While such inhibitors may also block signaling by other tyrosine kinase receptors involved in UB branching (e.g., FGF receptors), there is also data from specific mutations of the Ret receptor that implicates these pathways in the response to GDNF: an amino acid substitution for tyrosine 1062 of Ret9 (one of the two major Ret isoforms), which blocks activation of both Erk MAP kinase and PI3-kinase signaling in response to GDNF, results in severe defects in UB branching *in vivo* (Wong et al., 2005; Jain et al., 2006), as does a substitution for Ret tyrosine 1015, which prevents activation of the PLC-γ pathway (Jain et al., 2006).

One of the major consequences of GDNF/Gfrα1/Ret signaling is presumably the transcriptional activation of a set of "target" genes, but only a few of these targets in UB cells have been identified. One is the Ret gene itself, indicating a positive feedback loop that presumably reinforces the tip-specific expression pattern of Ret (Pepicelli et al., 1997). A second is Wnt11, another UB tip-specific gene that functions in the same positive feedback loop (Kispert et al., 1996; Pepicelli et al., 1997). Loss of Wnt11 results in reduced levels of GDNF in the MM and a mild reduction in UB branching, suggesting that Wnt11 signaling to the MM helps to maintain GDNF expression (Majumdar et al., 2003). A third target gene is Sprouty1 (Spry1), an intracellular negative regulator of tyrosine kinase signaling, which generates a negative feedback loop to regulate UB outgrowth and branching (Basson et al., 2005). Spry1 $-/-$ embryos often develop multiple ectopic UBs and multiplex kidneys, and a two-fold reduction of the GDNF gene dosage rescues these defects, revealing that Spry1 is needed to regulate budding by the WD in response to GDNF (Basson et al., 2005). Spry1 function in the UB

is also important to regulate later UB branching, as Spry1 $-/-$ embryos (or those with UB-specific knockout of Spry1) display an irregular pattern of branching with an increased tip number, apparently due to misexpression of Wnt11 in the UB trunks and increased GDNF expression in the MM.[1] In addition, expression of human Sprouty2 in the UB of transgenic mice caused decreased UB branching (Chi et al., 2004). It is not yet clear which signaling pathway(s) downstream of Ret is regulated by Spry1 in the UB, as Sprouty proteins can negatively regulate the ERK MAP kinase pathway as well as the PLC-γ and PI3-K pathways in response to FGF signaling (Sivak et al., 2005; Edwin et al., 2006; Mason et al., 2006).

What are the specific responses of UB cells that allow GDNF/Gfrα1/Ret signaling to exert its effects on both UB outgrowth and subsequent branching? Among the cellular processes discussed above that might mediate epithelial branching, GDNF has been implicated in two of them, proliferation and migration (and might also have effects on UB cell–cell adhesion, Sainio et al., 1997). GDNF beads placed next to a cultured kidney cause the expansion of the adjacent UB tips, apparently due to increased UB cell proliferation (Pepicelli et al., 1997; Michael and Davies, 2004). This is consistent with the mitogenic properties of GNDF for other cell types (Hu et al., 1999; Taraviras et al., 1999). Proliferation appears to be an important factor in the expansion of the ampulla, but whether GDNF-driven proliferation drives the subsequent branching of the ampulla is unclear. To do so, it would have to be very local proliferation, while the expression of GDNF surrounding the UB tips (at least at the mRNA level) is rather diffuse (Hellmich et al., 1996). This makes such a model unlikely, unless the distribution of secreted GDNF protein were much more localized. GDNF has also been implicated as a chemoattractive factor for migrating enteric neural crest cells (Young et al., 2001; Natarajan et al., 2002) and for cultured kidney cells (Tang et al., 1998), and it has been proposed that GNDF, via a related mechanism, guides the direction of growth of UB tips (Sariola and Saarma, 2003). However, again, the distribution of GDNF protein would have to be more highly localized than the GDNF mRNA distribution to serve such a role.

An experiment specifically designed to test the role of GNDF as a chemoattractive cue for UB morphogenesis failed to provide evidence for such a model. In this study, GDNF was misexpressed throughout the WD and UB of transgenic mice, in the presence or absence of endogenous GDNF (Shakya et al., 2005a). GDNF misexpression in the WD caused the outgrowth of numerous ectopic buds, many of which branched repeatedly outside of the kidney (supporting the idea that GDNF somehow promotes epithelial branching) (Figs. 3E,3F). However, even in the absence of endogenous, mesenchymal GDNF, the expression of GDNF in the UB epithelium was sufficient to promote nearly normal kidney development, including the characteristic bifurcation of UB tips at the periphery of the growing kidney (Shakya et al., 2005a). Therefore, if GDNF does serve as a chemoattractive factor for UB tips (which remains a possibility), it must be largely redundant with other chemoattractive factors.

Additional insight into the role of GDNF/Ret signaling has been obtained from studies in which ES cells homozygous for a Ret null mutation, and also carrying the Hoxb7/GFP transgene, were injected into wild-type blastocysts to generate chimeric embryos (Shakya et al., 2005b). In the genetically mosaic kidneys that resulted, the GFP marker could be used to follow the ability of the Ret $-/-$ cells to participate in WD and UB morphogenesis. While Ret $-/-$ cells were able to contribute extensively to the WD and to the trunk of the primary UB, they were specifically excluded from the tip of the primary UB at E11.0 (which was composed of only wild-type cells) (Figs. 6A,6B). At the T-stage, the mutant cells had progressed into the trunks of the secondary branches, but were excluded from the two new tips, and at later stages they were found in the trunks of several generations of branches, but never in the tips (Figs. 6C–6F). Thus, Ret is required for UB cells to contribute to the tip domain. One simple explanation may be that GDNF-driven UB cell proliferation is responsible for the formation of the tip domain, and thus, cells lacking Ret are unable to participate, but other models (such as a requirement for Ret for cell migration into the tip) cannot be excluded without further studies (Shakya et al., 2005b).

Another observation from these studies, which is relevant to the mechanism of UB branching, was that in the secondary UB branches, the Ret $-/-$ cells were only located on one side of the epithelial tube, the side nearest to the parental branch (Figs. 6C,6D). The simplest explanation for this non-random distribution is that new branches arise from the remodeling of the ampulla together with an adjacent segment of UB trunk, rather than simply from the ampulla. In this model, cells from the ampulla (tip cells) give rise to the new generation of tips and to the distal side of the trunks (consistent with the conclusion, mentioned above, that tip cells are bipotential) while the trunk cells generate the proximal side of the new branches (Fig. 7).

FGFs: While numerous members of the FGF family are expressed in the developing kidney (Cancilla et al., 2001; Chi et al., 2004), FGF7 and FGF10 have been

[1]Basson, A. M., Watson-Johnson, J., Shakya, R., Akbulut, S., Hyink, D., Costantini, F.D., Wilson, P.D., Mason, I.J., and Licht, J., Branching morphogenesis of the ureteric epithelium during kidney development is coordinated by the opposing functions of GDNF and Sprouty1. Manuscript submitted.

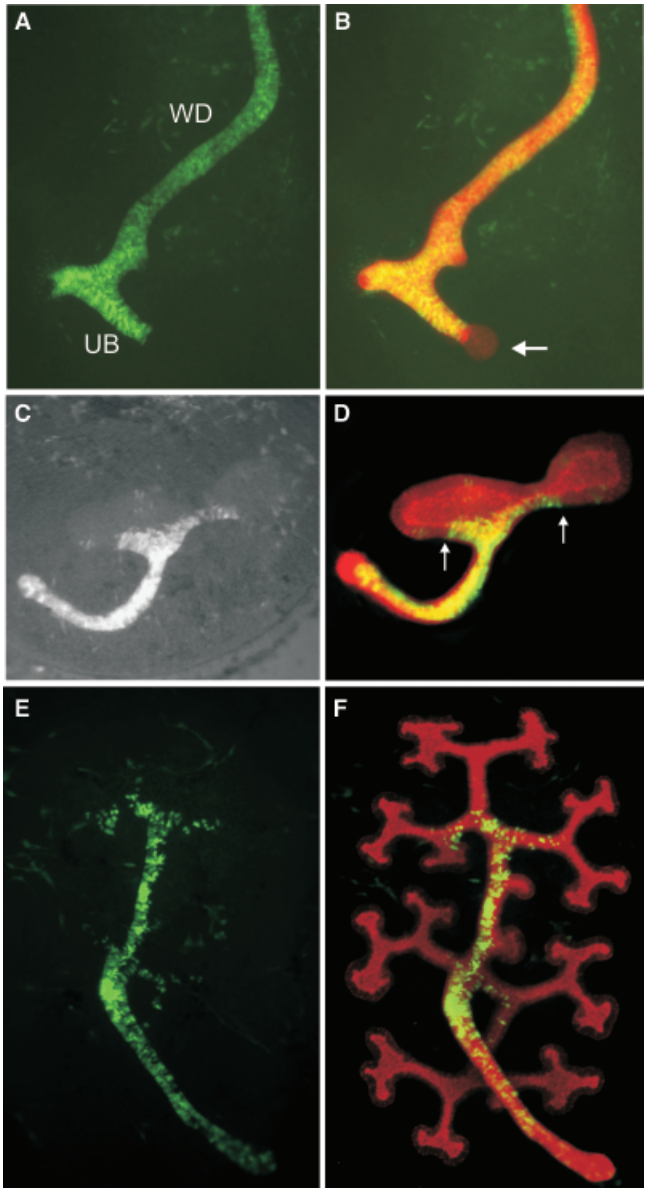

Fig. 6 Inability of Ret − / − cells to contribute to ureteric bud (UB) tips in chimeric kidneys. Ret − / − ES cells carrying the Hoxb7/green fluorescent protein (GFP) transgene were injected into wild-type blastocysts to examine their developmental potential in the kidneys of chimeric mice. (**A**) and (**E**) show GFP florescence, (**C**) shows superimposed bright field and GFP, and (**B**), (**D**) and (**F**) are counterstained with anti-cytokeratin (red) to show the entire Wolffian duct (WD)/UB epithelium. A-B, At E11.0 mutant cells (green) have contributed extensively to the Wolffian duct and the trunk of the primary UB, but not to the tip (arrow). C–D, At E11.5, Ret − / − cells contribute to the epithelium of the two secondary UB branches, on the side proximal to the ureter (arrows), but not to the tips or to the distal side of the branches. E-F, In an E11.5 kidney cultured for 38 hr, the mutant cells fail to contribute to the distal branches or tips. For experimental details, see Shakya et al. (2005b).

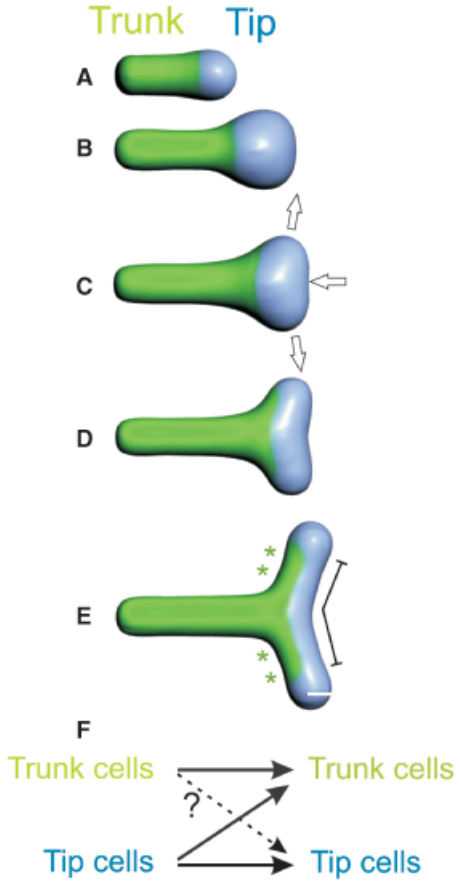

Fig. 7 A model for cell lineage during ureteric bud (UB) branching, based on the behavior of wild-type green fluorescent protein (GFP)+cells (e.g., Fig. 5) and Ret − / − cells (e.g., Fig. 6) in chimeric kidneys. (**A**) A UB branch contains trunk (green) and tip (blue) domains. (**B**), The trunk first elongates without acquiring tips cells, while the tip grows, stimulated by GDNF/Ret signaling, to form the ampulla. As branching ensues (**C** and **D**), the ampulla and the adjacent trunk epithelium are remodeled by hypothetical forces (arrows in C), causing the trunk epithelium to form the proximal side of two new trunks (asterisks in **E**), while tip cells form the two new tips and the distal epithelium of the new trunks (black bracket). (**F**), Summary of lineage relationships between tip and trunk cells; tip cells generate both tip and trunk cells, while trunk cells generate only more trunk cells during terminal branching (but presumably can form tip cells during lateral branching). (**A**)–(**E**) are modified from Shakya et al. (2005b) by permission of Elsevier.

most strongly implicated in UB branching morphogenesis (Qiao et al., 1999b, 2001; Ohuchi et al., 2000). These two FGFs both bind with high affinity to the FGFR2b (FGF receptor 2b) (Zhang et al., 2006), a receptor tyrosine kinase that (within the kidney) is expressed primarily or exclusively in the UB epithelium (Qiao et al., 2001; Zhao et al., 2004). FGF7 mRNA was first detected starting at E14.5 in the stroma surrounding the ureter and collecting ducts (Qiao et al., 1999b), and FGF10 mRNA was seen in medullary stroma at E15.5 (but in an ill-defined pattern at earlier stages) (D. Herzlinger and R.Guillaume, personal communication). The kidneys of mice lacking either FGF7 or FGF10 are slightly smaller than normal (Qiao et al., 1999b; Ohuchi et al., 2000), with reduced nephron number in the case of FGF7 (Qiao et al., 1999b). Because of their similar receptor-binding properties and overlapping expression,

FGF7 and FGF10 are likely to be partially redundant for kidney development *in vivo*, but the phenotype of double FGF7/FGF10 mutant mice has not yet been reported.

The effects of FGFs on UB morphogenesis have also been examined in organ culture. Transient addition of FGF7 to intact kidney organ cultures increased UB growth and branching as well as nephron number, while in contrast, prolonged exposure caused dilation and delayed differentiation of the UB (Qiao et al., 1999b). In the isolated UB system, FGF7 caused the UB to branch in a compact manner without clear stalks or ampullae, while FGF10 stimulated the formation of long stalks with distinct ampullae (Qiao et al., 2001) (Fig. 4). Thus, these two FGFs are implicated by both gain- and loss-of-function studies in UB morphogenesis, and may promote different aspects of branching morphogenesis.

Genetic manipulation of the FGFR2b receptor isoform has also revealed an important role for FGFs in this process. Transgenic mice that expressed a soluble dominant negative from of FGFR2b displayed agenesis or severe dysgenesis of the kidney (Celli et al., 1998). While FGFR2 $-/-$ embryos die before kidney development, recently a UB-specific knock-out of a conditional FGFR2 allele was carried out using a Hoxb7/Cre transgene (Zhao et al., 2004). While renal agenesis was not observed, the kidneys of the mutant mice were considerably smaller than normal, with thin UB stalks, reduced and aberrant UB branching, and reduced nephron number. In contrast, knock-out of FGFR1 in the UB had no effect on kidney development, and the double Fgfr1/2 UB knock-out was no more severe than FGFR2 alone (Zhao et al., 2004). These results clearly implicate FGFs as an important class of growth factors for UB branching morphogenesis. Because FGFs and GDNF signal through related tyrosine kinase receptors and activate many of the same downstream pathways (Eswarakumar et al., 2005), it is likely that there is some degree of redundancy between GDNF and FGF signaling during kidney development. Whether one of the FGFs could also be involved in stimulating the outgrowth of the UB from the WD (and perhaps account for the occasional UB formation in GDNF $-/-$ or Ret $-/-$ mice) remains to be tested.

Several other members of the FGF family, including FGFs 1, 2, 3, 4, 5, 6, 8, and 9 are expressed in the developing kidney (Qiao et al., 1999b, 2001; Cancilla et al., 2001), but none of the knock-outs aside from FGF7 and FGF10 cause obvious renal developmental defects, likely due to redundancy. Targeted inactivation of FGF8 in the MM lineage using Pax3-Cre, or using T-Cre which functions throughout the mesoderm, showed that FGF8 is required for survival of nephron progenitors and formation of S-shaped bodies by nascent nephrons and, but there is no evidence for a direct effect of FGF8 on the UB (Grieshammer et al., 2005; Perantoni et al., 2005).

TGFβ superfamily members

BMPs: Several members of the BMP family have been implicated in UB branching morphogenesis. BMPs 2, 3, 4, 5, 6 and 7 are all normally expressed in the developing kidney in distinct but partially overlapping patterns, as are their receptors (Dudley and Robertson, 1997; Godin et al., 1999; Martinez et al., 2001; Simic and Vukicevic, 2005). Of these, BMP4 is most clearly involved in regulating UB budding from the WD and subsequent branching.

BMP4 is expressed in stromal cells surrounding the WD before UB outgrowth and the stalk of the primary UB once it emerges, and later surrounding the collecting ducts, as well as in nascent nephrons (Dudley and Robertson, 1997). The BMP receptor subunit Alk3 (BMPR-1A) is expressed in both the WD/UB lineage and in the mesenchyme, while Alk6 (BMPR-1B) is specific to the WD and UB (Miyazaki et al., 2000; Raatikainen-Ahokas et al., 2000; Martinez et al., 2001). While BMP4 $-/-$ embryos die before kidney development (Winnier et al., 1995), heterozygotes sometimes display ectopic or duplicated UBs, which lead to ureteral and renal abnormalities inlcuding hydroureter, double ureter, and hypodysplastic kidneys (Miyazaki et al., 2000, 2003). The abnormal sites of ureteric budding in these mice led to the proposal that BMP4 may normally suppress ectopic bud formation from the WD (Miyazaki et al., 2000, 2003).

Studies in organ culture lent support to this model, and also showed that BMP4 can inhibit subsequent UB branching. BMP4 blocks the ability of GDNF to induce ectopic budding from the WD (Brophy et al., 2001), and BMP4-soaked beads placed next to the kidney inhibit nearby UB branching (Miyazaki et al., 2000). Addition of BMP4 to the culture medium also reduces UB branching, primarily in the posterior portion of the kidney, suggesting a role in the anterior-posterior patterning of the kidney (Raatikainen-Ahokas et al., 2000; Martinez et al., 2002; Cain et al., 2005). The target cells for these inhibitory effects were unclear, as BMP4 receptors are expressed both in the WD/UB and in the mesenchyme. However, this issue was clarified by experiments in which isolated UBs, free of mesenchyme, were cultured with BMP4 or other members of the TGFβ family (Bush et al., 2004). BMP4 strongly inhibited the growth and branching of the isolated UB, demonstrating that this factor is *capable* of directly affecting UB branching morphogenesis. Additional support for this conclusion comes from a study in which a constitutively active Alk3 receptor was expressed specifically in the UB lineage in transgenic mice. This caused a decrease in UB branching at E13.5 and renal aplasia, dysgenesis or renal medullary cystic dysplasia (with a decreased number of collecting ducts) in adult transgenic mice (Hu et al., 2003). These *in vitro* and *in vivo* results indicate that increased BMP4 or Alk3 signaling

can inhibit UB branching, while the defects in some BMP4+/− mice indicate that normal levels of BMP4 are *needed* to regulate UB outgrowth. Further manipulation of BMP4 expression or BMP signal transduction, though conditional gene targeting or other transgenic manipulations is needed to further elucidate the role of BMP4 in UB branching morphogenesis.

Another source of insight into this issue has been the analysis of mutations in Gremlin (Grem1), a cysteine knot protein that preferentially antagonizes BMP2 and BMP4 (and weakly BMP7). At E10, Gremlin is normally expressed in the posterior WD and in the surrounding MM. In Grem1−/− embryos, UB outgrowth is completely blocked, resulting in renal agenesis (Michos et al., 2004), and it was therefore postulated that Gremlin normally functions to block the inhibitory effect of a BMP on UB outgrowth. BMP2 is unlikely to be the relevant target of Gremlin in this situation, as it is expressed only later in the nascent nephrons, while BMP7 has no apparent role in UB outgrowth or early branching *in vivo*, so BMP4 is the likely target of Gremlin at this stage (Michos et al., 2004). Furthermore, recent genetic studies have revealed that a reduction of BMP4 gene dosage overcomes the absence of Grem1 and restores kidney development.[2]

Several other BMPs may also participate in later phases of UB branching morphogenesis, although the evidence is less conclusive. BMP2 can inhibit UB growth and branching in kidney cultures (Piscione et al., 1997) or isolated UBs (Bush et al., 2004). BMP2−/− embryos die before the stage of kidney development (Zhang and Bradley, 1996), and while BMP2+/− mice have apparently normal kidneys (Martinez et al., 2001; Martinez et al., 2002), a slight increase in UB cell proliferation and UB branching has been observed (Hartwig et al., 2005). BMP7 can stimulate branching at low concentrations but inhibits it at higher concentrations (Piscione et al., 1997). From loss-of-function studies, however, the best-documented role of BMP7 (which is expressed in many renal epithelia, including the UB and nascent nephrons) is to promote survival of the metanephric mesenchyme (Dudley and Robertson, 1997; Dudley et al., 1999); while BMP7−/− mutant kidneys also show reduced UB branching, this may be an indirect effect of the mesenchymal defects (Dudley et al., 1995; Godin et al., 1999).

Given the different activities of various BMPs on the UB in these assays, it was surprising that BMP4 was able to fully substitute for BMP7 during kidney development *in vivo*, in a gene-replacement experiment in which BMP4 was knocked into the BMP7 locus (Oxburgh et al., 2005). BMPs 4 and 7 are only about 30% identical and bind preferentially to different Type I receptors. These results suggest that the critical parameter may be the level of total BMPs in a particular domain, and that either BMP4 or BMP7 can activate the same signaling pathways necessary for MM survival (Oxburgh et al., 2005).

Activin and TGFβs: Other members of the TGFβ family may also play inhibitory roles in UB outgrowth and branching, based mainly on organ culture studies. A recent study showed that Activin A, which is produced by the WD as well as the surrounding mesonephros, may help to suppress ectopic UB formation (Maeshima et al., 2006). A neutralizing Activin A antibody potentiated the bud-inducing activity of GDNF beads on the WD; furthermore, when the mesonephric tissue was removed from the WD, GDNF was unable to induce budding from the isolated WD, except when the activity of endogenous Activin was blocked using the natural antagonist Follistatin, or Activin antibodies or siRNA. Thus, bud formation appears to be negatively regulated by autocrine/paracrine Activin A signals as well as the paracrine activity of BMP4 (Maeshima et al., 2006).

During kidney development, both TGFβ1 and TGFβ2 inhibit UB growth and/or branching in organ cultures (Rogers et al., 1993; Ritvos et al., 1995; Clark et al., 2001; Martinez et al., 2001). It was noted that they specifically delayed the primary UB branching, and increased the distances between branch points (Ritvos et al., 1995), which, interestingly, is similar to the effect of inhibiting Erk MAP kinase signaling (Fisher et al., 2001; Watanabe and Costantini, 2004). Activin A also decreased UB branching, although the effects appeared qualitatively somewhat different than those of TGFβ (Ritvos et al., 1995). When added to the isolated UB, TGFβ1 and Activin A (like BMP2 and BMP4) also strongly inhibited its growth and branching, apparently by reducing cell proliferation, demonstrating that they are capable of acting directly on the UB epithelium. Interestingly, in the presence of added FGF7 (which itself inhibited stalk elongation) all the TGFβ family members tested promoted elongation and thinning of the stalks, supporting the idea that the ultimate shape of the branched UB is molded by a combination of positive and negative regulators, which may act on different regions of the UB (Bush et al., 2004).

Of the TGFβ1, 2 and 3 knock-out mice, only TGFβ2−/− mice have kidney defects (Maeshima et al., 2001), including incompletely penetrant renal agenesis in females, as well as dilation of the renal pelvis and deterioration of ureteric epithelium after E15.5 (Sanford et al., 1997). Similarly, no renal defects have been reported in mice mutant for the various Activin β subunits (Martinez et al., 2001). Therefore, there is likely some redundancy between the different TGFβs

[2]Michos, O., Naillat, F., Gonçalves, A, Lopez-Rios, J., Beier, K., Vainio, S., and Zeller, R. Gremlin1-mediated BMP4 antagonism is key to initiate ureteric bud outgrowth and branching during metanephric kidney organogenesis. Manuscript submitted.

and the Activin β subunits in the control of UB morphogenesis.

Given the evidence for activities of TGFβ superfamily members on UB branching, it was quite surprising that the targeted deletion of Smad4 (the unique co-Smad required for Smad-dependent transcriptional regulation by BMPs, TGFβs, and Activins) in the UB lineage had no apparent effect on UB outgrowth or kidney development, at least up to E16.5 (Oxburgh et al., 2004). This indicates that signaling through the Smad4-dependent pathway is not *required* in the WD/UB lineage to control normal UB budding and branching, and that alternative signaling pathways must be at play. Smad4 could not be detected in the UB (by either RT-PCR or immunostaining) before E11.75, consistent with a Smad4-independent mechanism for the effects of BMP4 on budding and early branching (Oxburgh et al., 2004). However, it remains possible that some of the effects of *overexpressing* BMPs on UB branching in organ culture or in transgenic mice may be Smad4-dependent. Several examples of "non-canonical," Smad4-independent BMP signaling have been observed in the kidney. For example, constitutive Alk3 signaling in renal collecting ducts can activate the β-catenin/Tcf pathway, leading to elevated Myc expression (Hu and Rosenblum, 2005). In addition, low doses of BMP7, which stimulate UB branching in organ culture, can activate p38-MAP kinase in renal cell lines, through an unknown Smad1-independent mechanism (Hu et al., 2004).

Concluding comments

As this review should have made clear, while considerable progress has been made in recent years, understanding how UB branching morphogenesis is controlled remains a formidable challenge.

We now have the ability to visualize early UB branching in kidney cultures, but technical improvements are needed. These include the ability to better visualize the shapes and movements of individual cells within the UB, which may be achieved using different fluorescent proteins (e.g., to label cell membranes, cytoskeleton, or nuclei); fluorescent markers of other "colors" to trace different cell lineages, which can be used to follow the interactions between the UB, nascent nephrons, stromal cells, and other lineages; and better organ culture systems, which would allow 3D growth of the explanted kidney and faithful development to later stages. Improvements in the methods for culturing isolated UBs, such as the ability to grow the UB in fully defined culture media, would help to identify factors that act directly on the UB epithelium to mediate branching morphogenesis.

Methods for germline genetic manipulation are already quite powerful, but will be greatly expanded by the development of additional transgenic lines expressing Cre or Flp recombinases in specific renal cell types; this should be increasingly feasible as additional genes with specific patterns of expression (which can be used to drive the recombinase gene) are discovered by efforts such as GUDMAP. Equally important is the development of methods to bypass the germline and manipulate gene expression directly in organ cultures, and particularly methods that would allow genes to be directed to *specific locations* within the UB epithelium or surrounding cells. Branching appears to be initiated by changes in the behavior of small groups of cells in localized regions of the UB, which may communicate with adjacent groups of cells, and to understand this process, the ability to manipulate small and precisely localized groups of cells would be very powerful. Studies of the ability of embryonic stem cells (or possibly endogenous renal stem cells, Oliver, 2004; Steer and Nigam, 2004) to contribute to developing kidneys may open a different route to manipulate UB branching.

Identifying the specific cellular and subcellular mechanisms that drive UB branching morphogenesis remains a critical goal. In this area, there is clearly a great deal to be learned by comparison of branching morphogenesis in the kidney with that in other mammalian organs, as well as lower organisms. It seems likely that the cellular mechanisms underlying such a basic and widespread process are likely to be conserved during evolution, but there are also undoubtedly important organ-specific differences (Davies, 2002). Experimental methods that allow specific processes (e.g., cell proliferation, motility, changes in shape) to be manipulated in individual cells within the UB epithelium would be a significant aid in sorting out the important cellular events. One major area not covered in this review, which clearly impacts on branching morphogenesis, is the interaction of the UB epithelium with the extracellular matrix (for reviews, see Pohl et al., 2000; Davies, 2002; Steer and Nigam, 2004)

UB growth and branching are controlled by signals produced by several types of surrounding cells, and probably also those between different regions of the epithelium, and identifying these signals is a critical goal. In addition to the families of growth factors discussed in this review, numerous other secreted proteins have been implicated, and the pattern of growth and branching is undoubtedly the product of an extremely complex regulatory network of growth factors. Defining the normal patterns of expression of all the growth relevant factors and receptors during kidney development is an important descriptive step, but both gain- and loss-of-function studies will be needed to determine which of these are most important. A particularly significant question is how these signals pattern the UB; how do they determine the direction of growth of a UB branch, when it will elongate or branch again, and when it will stop growing? Again, the ability to manipulate the

expression of such growth factors in a spatially precise way seems to be an important step in figuring out which factors are important in this process, and how they function. As individual factors with significant functions are identified (e.g., GDNF and BMP4), the challenge will be to identify the specific intracellular networks that transmit these signals within UB cells, and the molecular responses, including the expression of target genes, regulated by each signal. Finally, these responses must be connected to the cellular behaviors that carry out morphogenetic program.

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References

Abrahamson, D.R. and Steenhard, B.M. (2006) Renal therapy by stem cells: outsource or in-house? J Am Soc Nephrol 17:12–14.

Al-Awqati, Q. and Goldberg, M.R. (1998) Architectural patterns in branching morphogenesis in the kidney. Kidney Int 54:1832–1842.

Arighi, E., Borrello, M.G. and Sariola, H. (2005) RET tyrosine kinase signaling in development and cancer. Cytokine Growth Factor Rev 16:441–467.

Arman, E., Haffner-Krausz, R., Chen, Y., Heath, J.K. and Lonai, P. (1998) Targeted disruption of fibroblast growth factor (FGF) receptor 2 suggests a role for FGF signaling in pregastrulation mammalian development. Proc Natl Acad Sci USA 95:5082–5087.

Baloh, R.H., Tansey, M.G., Golden, J.P., Creedon, D.J., Heuckeroth, R.O., Keck, C.L., Zimonjic, D.B., Popescu, N.C., Johnson, E.M. Jr. and Milbrandt, J. (1997) TrnR2, a novel receptor that mediates neurturin and GDNF signaling through Ret. Neuron 18:793–802.

Basson, M.A., Akbulut, S., Watson-Johnson, J., Simon, R., Carroll, T.J., Shakya, R., Gross, I., Martin, G.R., Lufkin, T., McMahon, A.P., Wilson, P.D., Costantini, F.D., Mason, I.J. and Licht, J.D. (2005) Sprouty1 is a critical regulator of GDNF/RET-mediated kidney induction. Dev Cell 8:229–239.

Batourina, E., Choi, C., Paragas, N., Bello, N., Hensle, T., Costantini, F.D., Schuchardt, A., Bacallao, R.L. and Mendelsohn, C.L. (2002) Distal ureter morphogenesis depends on epithelial cell remodeling mediated by vitamin A and Ret. Nat Genet 32:109–115.

Batourina, E., Gim, S., Bello, N., Shy, M., Clagett-Dame, M., Srinivas, S., Costantini, F. and Mendelsohn, C. (2001) Vitamin A controls epithelial/mesenchymal interactions through Ret expression. Nat Genet 27:74–78.

Batourina, E., Tsai, S., Lambert, S., Sprenkle, P., Viana, R., Dutta, S., Hensle, T., Wang, F., Niederreither, K., McMahon, A.P., Carroll, T.J. and Mendelsohn, C.L. (2005) Apoptosis induced by vitamin A signaling is crucial for connecting the ureters to the bladder. Nat Genet 37:1082–1089.

Belteki, G., Haigh, J., Kabacs, N., Haigh, K., Sison, K., Costantini, F., Whitsett, J., Quaggin, S.E. and Nagy, A. (2005) Conditional and inducible transgene expression in mice through the combinatorial use of Cre-mediated recombination and tetracycline induction. Nucleic Acids Res 33:e51.

Bianco, R.A., Keen, H.L., Lavoie, J.L. and Sigmund, C.D. (2003) Untraditional methods for targeting the kidney in transgenic mice. Am J Physiol Renal Physiol 285:F1027–F1033.

Bouchard, M. (2004) Transcriptional control of kidney development. Differentiation 72:295–306.

Brophy, P.D., Ostrom, L., Lang, K.M. and Dressler, G.R. (2001) Regulation of ureteric bud outgrowth by Pax2-dependent activation of the glial derived neurotrophic factor gene. Development 128:4747–4756.

Bush, K.T., Sakurai, H., Steer, D.L., Leonard, M.O., Sampogna, R.V., Meyer, T.N., Schwesinger, C., Qiao, J. and Nigam, S.K. (2004) TGF-beta superfamily members modulate growth, branching, shaping, and patterning of the ureteric bud. Dev Biol 266:285–298.

Cabernard, C. and Affolter, M. (2005) Distinct roles for two receptor tyrosine kinases in epithelial branching morphogenesis in Drosophila. Dev Cell 9:831–842.

Cacalano, G., Farinas, I., Wang, L.C., Hagler, K., Forgie, A., Moore, M., Armanini, M., Phillips, H., Ryan, A.M., Reichardt, L.F., Hynes, M., Davies, A. and Rosenthal, A. (1998) GFRalpha1 is an essential receptor component for GDNF in the developing nervous system and kidney. Neuron 21:53–62.

Cain, J.E., Nion, T., Jeulin, D. and Bertram, J.F. (2005) Exogenous BMP-4 amplifies asymmetric ureteric branching in the developing mouse kidney in vitro. Kidney Int 67:420–431.

Cancilla, B., Davies, A., Cauchi, J.A., Risbridger, G.P. and Bertram, J.F. (2001) Fibroblast growth factor receptors and their ligands in the adult rat kidney. Kidney Int 60:147–155.

Carroll, T.J. and McMahon, A.P. (2003) Overview: the molecular basis of kidney development. In: Vize, P.D., Woolf, A.S. and Bard, J.B.L. eds. The kidney. From normal development to congenital disease. Academic Press, Amsterdam, pp. 343–376.

Caruana, G., Cullen-McEwen, L., Nelson, A.L., Kostoulias, X., Woods, K., Gardiner, B., Davis, M.J., Taylor, D.F., Teasdale, R.D., Grimmond, S.M., Little, M.H. and Bertram, J.F. (2006a) Spatial gene expression in the T-stage mouse metanephros. Gene Expr Patterns.

Caruana, G., Young, R.J. and Bertram, J.F. (2006b) Imaging the embryonic kidney. Nephron Exp Nephrol 103:e62–e68.

Cebrian, C., Borodo, K., Charles, N. and Herzlinger, D.A. (2004) Morphometric index of the developing murine kidney. Dev Dyn 231:601–608.

Celli, G., LaRochelle, W.J., Mackem, S., Sharp, R. and Merlino, G. (1998) Soluble dominant-negative receptor uncovers essential roles for fibroblast growth factors in multi-organ induction and patterning. EMBO J 17:1642–1655.

Chi, L., Zhang, S., Lin, Y., Prunskaite-Hyyrylainen, R., Vuolteenaho, R., Itaranta, P. and Vainio, S. (2004) Sprouty proteins regulate ureteric branching by coordinating reciprocal epithelial Wnt11, mesenchymal Gdnf and stromal Fgf7 signalling during kidney development. Development 131:3345–3356.

Clark, A.T. and Bertram, J.F. (1999) Molecular regulation of nephron endowment. Am J Physiol 276:F485–F497.

Clark, A.T., Young, R.J. and Bertram, J.F. (2001) In vitro studies on the roles of transforming growth factor-beta 1 in rat metanephric development. Kidney Int 59:1641–1653.

Coles, H.S.R., Burne, J.F. and Raff, M.C. (1993) Large-scale normal cell death in the developing rat kidney and its reduction by epidermal growth factor. Development 118:777–784.

Costantini, F. and Shakya, R. (2006) GDNF/Ret signaling and the development of the kidney. Bioessays 28:117–127.

Cullen-McEwen, L.A., Caruana, G. and Bertram, J.F. (2005) The where, what and why of the developing renal stroma. Nephron Exp Nephrol 99:e1–e8.

Cullen-McEwen, L.A., Drago, J. and Bertram, J.F. (2001) Nephron endowment in glial cell line-derived neurotrophic factor (GDNF) heterozygous mice. Kidney Int 60:31–36.

Davies, J.A. (1999) The Kidney Development Database. Dev Genet 24:194–198.

Davies, J.A. (2002) Do different branching epithelia use a conserved developmental mechanism? Bioessays 24:937–948.

Davies, J.A., Ladomery, M., Hohenstein, P., Michael, L., Shafe, A., Spraggon, L. and Hastie, N. (2004) Development of an siRNA-based method for repressing specific genes in renal organ culture and its use to show that the Wt1 tumour suppressor is required for nephron differentiation. Hum Mol Genet 13:235–246.

de Graaff, E., Srinivas, S., Kilkenny, C., D'Agati, V., Mankoo, B.S., Costantini, F. and Pachnis, V. (2001) Differential activities of the RET tyrosine kinase receptor isoforms during mammalian embryogenesis. Genes Dev 15:2433–2444.

Dudley, A.T., Godin, R.E. and Robertson, E.J. (1999) Interaction between FGF and BMP signaling pathways regulates development of metanephric mesenchyme. Genes Dev 13:1601–1613.

Dudley, A.T., Lyons, K.M. and Robertson, E.J. (1995) A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. Genes Dev 9:2795–2807.

Dudley, A.T. and Robertson, E.J. (1997) Overlapping expression domains of bone morphogenetic protein family members potentially account for limited tissue defects in BMP7 deficient embryos. Dev Dyn 208:349–362.

Edwin, F., Singh, R., Endersby, R., Baker, S.J. and Patel, T.B. (2006) The Tumor suppressor PTEN is necessary for human sprouty 2-mediated inhibition of cell proliferation 10.1074/jbc.M508300200. J Biol Chem 281:4816–4822.

Ehrenfels, C.W., Carmillo, P.J., Orozco, O., Cate, R.L. and Sanicola, M. (1999) Perturbation of RET signaling in the embryonic kidney. Dev Genet 24:263–272.

Ekblom, P. (1992) Renal development. In: Seldin, D.W. and Giebisch, G. eds. The kidney: physiology and pathophysiology. Raven Press, New York, pp. 475–501.

Enomoto, H., Araki, T., Jackman, A., Heuckeroth, R.O., Snider, W.D., Johnson, E.M.J. and Milbrandt, J. (1998) GFRa1-deficient mice have deficits in the enteric nervous system and kidneys. Neuron 21:317–324.

Erickson, R.A. (1968) Inductive interactions in the development of the mouse metanephros. J Exp Zool 169:33–42.

Eswarakumar, V.P., Lax, I. and Schlessinger, J. (2005) Cellular signaling by fibroblast growth factor receptors. Cytokine Growth Factor Rev 16:139–149.

Ettensohn, C.A. (1985) Mechanisms of epithelial invagination. Quart Rev Biol 60:289–307.

Fisher, C.E., Michael, L., Barnett, M.W. and Davies, J.A. (2001) Erk MAP kinase regulates branching morphogenesis in the developing mouse kidney. Development 128:4329–4338.

Furth, P.A., St Onge, L., Boger, H., Gruss, P., Gossen, M., Kistner, A., Bujard, H. and Hennighausen, L. (1994) Temporal control of gene expression in transgenic mice by a tetracycline-responsive promoter. Proc Natl Acad Sci USA 91:9302–9306.

Gao, X., Chen, X., Taglienti, M., Rumballe, B., Little, M.H. and Kreidberg, J.A. (2005) Angioblast–mesenchyme induction of early kidney development is mediated by Wt1 and Vegfa. Development 132:5437–5449.

Gawlik, A. and Quaggin, S.E. (2004) Deciphering the renal code: advances in conditional gene targeting. Physiology (Bethesda) 19:245–252.

Godin, R.E., Robertson, E.J. and Dudley, A.T. (1999) Role of BMP family members during kidney development. Int J Dev Biol 43:405–411.

Gossen, M., Freundlieb, S., Bender, G., Muller, G., Hillen, W. and Bujard, H. (1995) Transcriptional activation by tetracyclines in mammalian cells. Science 268:1766–1769.

Grieshammer, U., Cebrian, C., Ilagan, R., Meyers, E., Herzlinger, D. and Martin, G.R. (2005) FGF8 is required for cell survival at distinct stages of nephrogenesis and for regulation of gene expression in nascent nephrons. Development 132:3847–3857.

Grobstein, C. (1953a) Inductive epithelio-mesenchymal interaction in cultured organ rudiments of the mouse. Science 118:52–55.

Grobstein, C. (1953b) Morphogenetic interaction between embryonic mouse tissues separated by a membrane filter. Nature 172:869–871.

Grobstein, C. (1955) Inductive interaction in the development of the mouse metanephros. J Exp Zool 130:319–340.

Hartwig, S., Hu, M.C., Cella, C., Piscione, T., Filmus, J. and Rosenblum, N.D. (2005) Glypican-3 modulates inhibitory Bmp2-Smad signaling to control renal development in vivo. Mech Dev 122:928–938.

Hatini, V., Huh, S.O., Herzlinger, D., Soares, V.C. and Lai, E. (1996) Essential role of stromal mesenchyme in kidney morphogenesis revealed by targeted disruption of Winged Helix transcription factor BF-2. Genes Dev 10:1467–1478.

Hellmich, H.L., Kos, L., Cho, E.S., Mahon, K.A. and Zimmer, A. (1996) Embryonic expression of glial cell-line derived neurotrophic factor (GDNF) suggests multiple developmental roles in neural differentiation and epithelial-mesenchymal interactions. Mech Dev 54:95–105.

Herzlinger, D., Abramson, R. and Cohen, D. (1993) Phenotypic conversions in renal development. J Cell Sci 17(Suppl):61–64.

Herzlinger, D., Koseki, C., Mikawa, T. and Al, A.Q. (1992) Metanephric mesenchyme contains multipotent stem cells whose fate is restricted after induction. Development 114:565–572.

Hogan, B.L. (1999) Morphogenesis. Cell 96:225–233.

Hu, M.C., Piscione, T.D. and Rosenblum, N.D. (2003) Elevated SMAD1/beta-catenin molecular complexes and renal medullary cystic dysplasia in ALK3 transgenic mice. Development 130:2753–2766.

Hu, M.C. and Rosenblum, N.D. (2005) Smad1, beta-catenin and Tcf4 associate in a molecular complex with the Myc promoter in dysplastic renal tissue and cooperate to control Myc transcription. Development 132:215–225.

Hu, J., Shima, H. and Nakagawa, H. (1999) Glial cell line-derived neurotropic factor stimulates sertoli cell proliferation in the early postnatal period of rat testis development. Endocrinology 140:3416–3421.

Hu, M.C., Wasserman, D., Hartwig, S. and Rosenblum, N.D. (2004) p38MAPK acts in the BMP7-dependent stimulatory pathway during epithelial cell morphogenesis and is regulated by Smad1. J Biol Chem 279:12051–12059.

Igarashi, P. (2004) Kidney-specific gene targeting. J Am Soc Nephrol 15:2237–2239.

Jain, S., Encinas, M., Johnson, E.M. Jr. and Milbrandt, J. (2006) Critical and distinct roles for key RET tyrosine docking sites in renal development. Genes Dev 20:321–333.

Jijiwa, M., Fukuda, T., Kawai, K., Nakamura, A., Kurokawa, K., Murakumo, Y., Ichihara, M. and Takahashi, M. (2004) A targeting mutation of tyrosine 1062 in Ret causes a marked decrease of enteric neurons and renal hypoplasia. Mol Cell Biol 24:8026–8036.

Keller, R. (2006) Mechanisms of elongation in embryogenesis. Development 133:2291–2302.

Kispert, A., Vainio, S., Shen, L., Rowitch, D.H. and McMahon, A.P. (1996) Proteoglycans are required for maintenance of Wnt-11 expression in the ureter tips. Development 122:3627–3637.

Kobayashi, A., Kwan, K.M., Carroll, T.J., McMahon, A.P., Mendelsohn, C.L. and Behringer, R.R. (2005) Distinct and sequential tissue-specific activities of the LIM-class homeobox gene Lim1 for tubular morphogenesis during kidney development. Development 132:2809–2823.

Kuschert, S., Rowitch, D.H., Haenig, B., McMahon, A.P. and Kispert, A. (2001) Characterization of Pax-2 regulatory sequences that direct transgene expression in the Wolffian duct and its derivatives. Dev Biol 229:128–140.

Levinson, R.S., Batourina, E., Choi, C., Vorontchikhina, M., Kitajewski, J. and Mendelsohn, C.L. (2005) Foxd1-dependent signals control cellularity in the renal capsule, a structure required for normal renal development. Development 132:529–539.

Levinson, R. and Mendelsohn, C. (2003) Stromal progenitors are important for patterning epithelial and mesenchymal cell types in the embryonic kidney. Semin Cell Dev Biol 14:225–231.

Li, X., Hyink, D.P., Polgar, K., Gusella, G.L., Wilson, P.D. and Burrow, C.R. (2005) Protein kinase X activates ureteric bud branching morphogenesis in developing mouse metanephric kidney. J Am Soc Nephrol 16:3543–3552.

Lin, Y., Zhang, S., Tuukkanen, J., Peltoketo, H., Pihlajaniemi, T. and Vainio, S. (2003) Patterning parameters associated with the branching of the ureteric bud regulated by epithelial–mesenchymal interactions. Int J Dev Biol 47:3–13.

Maeshima, A., Nojima, Y. and Kojima, I. (2001) The role of the activin-follistatin system in the developmental and regeneration processes of the kidney. Cytokine Growth Factor Rev 12:289–298.

Maeshima, A., Vaughn, D.A., Choi, Y. and Nigam, S.K. (2006) Activin A is an endogenous inhibitor of ureteric bud outgrowth from the Wolffian duct. Dev Biol 295:473–485.

Majumdar, A., Vainio, S., Kispert, A., McMahon, J. and McMahon, A.P. (2003) Wnt11 and Ret/Gdnf pathways cooperate in regulating ureteric branching during metanephric kidney development. Development 130:3175–3185.

Martinez, G., Loveland, K.L., Clark, A.T., Dziadek, M. and Bertram, J.F. (2001) Expression of bone morphogenetic protein receptors in the developing mouse metanephros. Exp Nephrol 9:372–379.

Martinez, G., Mishina, Y. and Bertram, J.F. (2002) BMPs and BMP receptors in mouse metanephric development: in vivo and in vitro studies. Int J Dev Biol 46:525–533.

Mason, J.M., Morrison, D.J., Basson, M.A. and Licht, J.D. (2006) Sprouty proteins: multifaceted negative-feedback regulators of receptor tyrosine kinase signaling. Trends Cell Biol 16: 45–54.

Mendelsohn, C., Batourina, E., Fung, S., Gilbert, T. and Dodd, J. (1999) Stromal cells mediate retinoid-dependent functions essential for renal development. Development 126:1139–1148.

Meyer, T.N., Schwesinger, C., Bush, K.T., Stuart, R.O., Rose, D.W., Shah, M.M., Vaughn, D.A., Steer, D.L. and Nigam, S.K. (2004) Spatiotemporal regulation of morphogenetic molecules during in vitro branching of the isolated ureteric bud: toward a model of branching through budding in the developing kidney. Dev Biol 275:44–67.

Michael, L. and Davies, J.A. (2004) Pattern and regulation of cell proliferation during murine ureteric bud development. J Anat 204:241–255.

Michael, L., Sweeney, D.E. and Davies, J.A. (2005) A role for microfilament-based contraction in branching morphogenesis of the ureteric bud. Kidney Int 68:2010–2018.

Michos, O., Panman, L., Vintersten, K., Beier, K., Zeller, R. and Zuniga, A. (2004) Gremlin-mediated BMP antagonism induces the epithelial-mesenchymal feedback signaling controlling metanephric kidney and limb organogenesis. Development 131:3401–3410.

Miyazaki, Y., Oshima, K., Fogo, A., Hogan, B.L. and Ichikawa, I. (2000) Bone morphogenetic protein 4 regulates the budding site and elongation of the mouse ureter. J Clin Invest 105: 863–873.

Miyazaki, Y., Oshima, K., Fogo, A. and Ichikawa, I. (2003) Evidence that bone morphogenetic protein 4 has multiple biological functions during kidney and urinary tract development. Kidney Int 63:835–844.

Moore, M.W., Klein, R.D., Farinas, I., Sauer, H., Armanini, M., Phillips, H., Reichardt, L.F., Ryan, A.M., Carver-Moore, K. and Rosenthal, A. (1996) Renal and neuronal abnormalities in mice lacking GDNF. Nature 382:76–79.

Natarajan, D., Marcos-Gutierrez, C., Pachnis, V. and de Graaff, E. (2002) Requirement of signalling by receptor tyrosine kinase RET for the directed migration of enteric nervous system progenitor cells during mammalian embryogenesis. Development 129:5151–5160.

Nogawa, H., Morita, K. and Cardoso, W.V. (1998) Bud formation precedes the appearance of differential cell proliferation during branching morphogenesis of mouse lung epithelium in vitro. Dev Dyn 213:228–235.

Novak, A., Guo, C., Yang, W., Nagy, A. and Lobe, C.G. (2000) Z/EG, a double reporter mouse line that expresses enhanced green fluorescent protein upon cre-mediated excision. Genesis 28: 147–155.

Ohuchi, H., Hori, Y., Yamasaki, M., Harada, H., Sekine, K., Kato, S. and Itoh, N. (2000) FGF10 acts as a major ligand for FGF receptor 2 IIIb in mouse multi-organ development. Biochem Biophys Res Commun 277:643–649.

Oliver, J. (1968) Nephrons and kidneys; a quantitative study of developmental and evolutionary mammalian renal architectonics. Hoeber Medical Division, Harper & Row, New York.

Oliver, J.A. (2004) Adult renal stem cells and renal repair. Curr Opin Nephrol Hypertens 13:17–22.

Osathanondh, V. and Potter, E. (1963a) Development of human kidney as shown by microdissection. I. Preparation of tissue with reasons for possible misinterpretations of observations. Arch Pathol 76:271–276.

Osathanondh, V. and Potter, E. (1963b) Development of human kidney as shown by microdissection. II. Renal Pelvis, calyces, and papillae. Arch Pathol 76:277–289.

Osathanondh, V. and Potter, E. (1963c) Development of human kidney as shown by microdissection. III. Formation and interrelationships of collecting tubules and nephrons. Arch Pathol 76:290–302.

Oxburgh, L., Chu, G.C., Michael, S.K. and Robertson, E.J. (2004) TGFbeta superfamily signals are required for morphogenesis of the kidney mesenchyme progenitor population. Development 131:4593–4605.

Oxburgh, L., Dudley, A.T., Godin, R.E., Koonce, C.H., Islam, A., Anderson, D.C., Bikoff, E.K. and obertson, E.J. (2005) BMP4 substitutes for loss of BMP7 during kidney development. Dev Biol 286:637–646.

Pachnis, V., Mankoo, B.S. and Costantini, F. (1993) Expression of the *c-ret* proto-oncogene during mouse embryogenesis. Development 119:1005–1017.

Patterson, L.T., Pembaur, M. and Potter, S.S. (2001) Hoxa11 and Hoxd11 regulate branching morphogenesis of the ureteric bud in the developing kidney. Development 128: 2153–2161.

Pepicelli, C.V., Kispert, A., Rowitch, D.H. and McMahon, A.P. (1997) GDNF induces branching and increased cell proliferation in the ureter of the mouse. Dev Biol 192:193–198.

Perantoni, A.O., Timofeeva, O., Naillat, F., Richman, C., Pajni-Underwood, S., Wilson, C., Vainio, S., Dove, L.F. and Lewandoski, M. (2005) Inactivation of FGF8 in early mesoderm reveals an essential role in kidney development. Development 132:3859–3871.

Perantoni, A.O., Williams, C.L. and Lewellyn, A.L. (1991) Growth and branching morphogenesis of rat collecting duct anlagen in the absence of metanephrogenic mesenchyme. Differentiation 48:107–113.

Pichel, J.G., Shen, L., Sheng, H.Z., Granholm, A.C., Drago, J., Grinberg, A., Lee, E.J., Huang, S.P., Saarma, M., Hoffer, B.J., Sariola, H. and Westphal, H. (1996) Defects in enteric innervation and kidney development in mice lacking GDNF. Nature 382:73–76.

Pilot, F. and Lecuit, T. (2005) Compartmentalized morphogenesis in epithelia: from cell to tissue shape. Dev Dyn 232:685–694.

Piscione, T.D. and Rosenblum, N.D. (2002) The molecular control of renal branching morphogenesis: current knowledge and emerging insights. Differentiation 70:227–246.

Piscione, T.D., Yager, T.D., Gupta, I.R., Grinfeld, B., Pei, Y., Attisano, L., Wrana, J.L. and Rosenblum, N.D. (1997) BMP-2 and OP-1 exert direct and opposite effects on renal branching morphogenesis. Am J Physiol 273:F961–F975.

Pohl, M., Bhatnagar, V., Mendoza, S.A. and Nigam, S.K. (2002) Toward an etiological classification of developmental disorders of the kidney and upper urinary tract. Kidney Int 61:10–19.

Pohl, M., Stuart, R.O., Sakurai, H. and Nigam, S.K. (2000) Branching morphogenesis during kidney development. Annu Rev Physiol 62:595–620.

Poladia, D.P., Kish, K., Kutay, B., Bauer, J., Baum, M. and Bates, C.M. (2006) Link between reduced nephron number and hypertension: studies in a mutant mouse model. Pediatr Res 59: 489–493.

Polgar, K., Burrow, C.R., Hyink, D.P., Fernandez, H., Thornton, K., Li, X., Gusella, G.L. and Wilson, P.D. (2005) Disruption of polycystin-1 function interferes with branching morphogenesis of the ureteric bud in developing mouse kidneys. Dev Biol 286: 16–30.

Qiao, J., Bush, K.T., Steer, D.L., Stuart, R.O., Sakurai, H., Wachsman, W. and Nigam, S.K. (2001) Multiple fibroblast growth factors support growth of the ureteric bud but have different effects on branching morphogenesis. Mech Dev 109: 123–135.

Qiao, J., Cohen, D. and Herzlinger, D. (1995) The metanephric blastema differentiates into collecting system and nephron epithelia in vitro. Development 121:3207–3214.

Qiao, J., Sakurai, H. and Nigam, S.K. (1999a) Branching morphogenesis independent of mesenchymal-epithelial contact in the developing kidney. Proc Natl Acad Sci USA 96: 7330–7335.

Qiao, J., Uzzo, R., Obara-Ishihara, T., Degenstein, L., Fuchs, E. and Herzlinger, D. (1999b) FGF-7 modulates ureteric bud growth and nephron number in the developing kidney. Development 126:547–554.

Raatikainen-Ahokas, A., Hytonen, M., Tenhunen, A., Sainio, K. and Sariola, H. (2000) BMP-4 affects the differentiation of metanephric mesenchyme and reveals an early anterior-posterior axis of the embryonic kidney. Dev Dyn 217:146–158.

Ritvos, O., Tuuri, T., Eramaa, M., Sainio, K., Hilden, K., Saxen, L. and Gilbert, S.F. (1995) Activin disrupts epithelial branching morphogenesis in developing glandular organs of the mouse. Mech Dev 50:229–245.

Rogers, S.A., Ryan, G., Purchio, A.F. and Hammerman, M.R. (1993) Metanephric transforming growth factor-beta 1 regulates nephrogenesis in vitro. Am J Physiol 264:F996–F1002.

Rosario, M. and Birchmeier, W. (2003) How to make tubes: signaling by the Met receptor tyrosine kinase. Trends Cell Biol 13:328–335.

Sainio, K., Suvanto, P., Davies, J., Wartiovaara, J., Wartiovaara, K., Saarma, M., Arumae, U., Meng, X., Lindahl, M., Pachnis, V. and Sariola, H. (1997) Glial-cell-line-derived neurotrophic factor is required for bud initiation from ureteric epithelium. Development 124:4077–4087.

Sakurai, H., Bush, K.T. and Nigam, S.K. (2001) Identification of pleiotrophin as a mesenchymal factor involved in ureteric bud branching morphogenesis. Development 128: 3283–3293.

Sanchez, M.P., Silos-Santiago, I., Frisen, J., He, B., Lira, S.A. and Barbacid, M. (1996) Renal agenesis and the absence of enteric neurons in mice lacking GDNF. Nature 382:70–73.

Sanford, L.P., Ormsby, I., Gittenberger-de Groot, A.C., Sariola, H., Friedman, R., Boivin, G.P., Cardell, E.L. and Doetschman, T. (1997) TGFbeta2 knockout mice have multiple developmental defects that are non-overlapping with other TGFbeta knockout phenotypes. Development 124:2659–2670.

Sariola, H. and Saarma, M. (2003) Novel functions and signalling pathways for GDNF. J Cell Sci 116:3855–3862.

Sariola, H. and Sainio, K. (1997) The tip-top branching ureter. Curr Opin Cell Biol 9:877–884.

Saxen, L. (1987) Organogenesis of the kidney. Cambridge University Press, Cambridge.

Schmidt-Ott, K.M., Yang, J., Chen, X., Wang, H., Paragas, N., Mori, K., Li, J.Y., Lu, B., Costantini, F., Schiffer, M., Bottinger, E. and Barasch, J. (2005) Novel regulators of kidney development from the tips of the ureteric bud. J Am Soc Nephrol 16:1993–2002.

Schuchardt, A., D'Agati, V., Larsson-Blomberg, L., Costantini, F. and Pachnis, V. (1994) Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret. Nature 367:380–383.

Schuchardt, A., D'Agati, V., Pachnis, V. and Costantini, F. (1996) Renal agenesis and hypodysplasia in ret-k- mutant mice result from defects in ureteric bud development. Development 122:1919–1929.

Schwab, K., Patterson, L.T., Aronow, B.J., Luckas, R., Liang, H.C. and Potter, S.S. (2003) A catalogue of gene expression in the developing kidney. Kidney Int 64:1588–1604.

Shah, M.M., Sampogna, R.V., Sakurai, H., Bush, K.T. and Nigam, S.K. (2004) Branching morphogenesis and kidney disease. Development 131:1449–1462.

Shakya, R., Jho, E.H., Kotka, P., Wu, Z., Kholodilov, N., Burke, R., D'Agati, V. and Costantini, F. (2005a) The role of GDNF in patterning the excretory system. Dev Biol 283:70–84.

Shakya, R., Watanabe, T. and Costantini, F. (2005b) The role of GDNF/Ret signaling in ureteric bud cell fate and branching morphogenesis. Dev Cell 8:65–74.

Simic, P. and Vukicevic, S. (2005) Bone morphogenetic proteins in development and homeostasis of kidney. Cytokine Growth Factor Rev 16:299–308.

Sivak, J.M., Petersen, L.F. and Amaya, E. (2005) FGF signal interpretation is directed by Sprouty and Spred proteins during mesoderm formation. Dev Cell 8:689–701.

Soriano, P. (1999) Generalized lacZ expression with the ROSA26 Cre reporter strain. Nat Genet 21:70–71.

Srinivas, S., Goldberg, M.R., Watanabe, T., D'Agati, V., al-Awqati, Q. and Costantini, F. (1999a) Expression of green fluorescent protein in the ureteric bud of transgenic mice: a new tool for the analysis of ureteric bud morphogenesis. Dev Genet 24:241–251.

Srinivas, S., Watanabe, T., Lin, C.S., William, C.M., Tanabe, Y., Jessell, T.M. and Costantini, F. (2001) Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. BMC Dev Biol 1:4.

Srinivas, S., Wu, Z., Chen, C.M., D'Agati, V. and Costantini, F. (1999b) Dominant effects of RET receptor misexpression and ligand-independent RET signaling on ureteric bud development. Development 126:1375–1386.

Steenhard, B.M., Isom, K.S., Cazcarro, P., Dunmore, J.H., Godwin, A.R., St John, P.L. and Abrahamson, D.R. (2005) Integration of embryonic stem cells in metanephric kidney organ culture. J Am Soc Nephrol 16:1623–1631.

Steer, D.L. and Nigam, S.K. (2004) Developmental approaches to kidney tissue engineering. Am J Physiol Renal Physiol 286: F1–F7.

Stuart, R.O., Bush, K.T. and Nigam, S.K. (2001) Changes in global gene expression patterns during development and maturation of the rat kidney. Proc Natl Acad Sci USA 98:5649–5654.

Stuart, R.O., Bush, K.T. and Nigam, S.K. (2003) Changes in gene expression patterns in the ureteric bud and metanephric mesenchyme in models of kidney development. Kidney Int 64:1997–2008.

Takahashi, M. (2001) The GDNF/RET signaling pathway and human diseases. Cytokine Growth Factor Rev 12:361–373.

Tang, M.J., Cai, Y., Tsai, S.J., Wang, Y.K. and Dressler, G.R. (2002) Ureteric bud outgrowth in response to RET activation is mediated by phosphatidylinositol 3-kinase. Dev Biol 243: 128–136.

Tang, M.J., Worley, D., Sanicola, M. and Dressler, G.R. (1998) The RET-glial cell-derived neurotrophic factor (GDNF) pathway stimulates migration and chemoattraction of epithelial cells. J Cell Biol 142:1337–1345.

Taraviras, S., Marcos-Gutierrez, C.V., Durbec, P., Jani, H., Grigoriou, M., Sukumaran, M., Wang, L.C., Hynes, M., Raisman, G.

and Pachnis, V. (1999) Signalling by the RET receptor tyrosine kinase and its role in the development of the mammalian enteric nervous system. Development 126:2785–2797.

Thivierge, C., Kurbegovic, A., Couillard, M., Guillaume, R., Cote, O. and Trudel, M. (2006) Overexpression of PKD1 causes polycystic kidney disease. Mol Cell Biol 26:1538–1548.

Towers, P.R., Woolf, A.S. and Hardman, P. (1998) Glial cell line-derived neurotrophic factor stimulates ureteric bud outgrowth and enhances survival of ureteric bud cells in vitro. Exp Nephrol 6:337–351.

Tufro-McReddie, A., Norwood, V.F., Aylor, K.W., Botkin, S.J., Carey, R.M. and Gomez, R.A. (1997) Oxygen regulates vascular endothelial growth factor-mediated vasculogenesis and tubulogenesis. Dev Biol 183:139–149.

Urlinger, S., Baron, U., Thellmann, M., Hasan, M.T., Bujard, H. and Hillen, W. (2000) Exploring the sequence space for tetracycline-dependent transcriptional activators: novel mutations yield expanded range and sensitivity. Proc Natl Acad Sci USA 97:7963–7968.

Vainio, S.J. (2003) Nephrogenesis regulated by Wnt signaling. J Nephrol 16:279–285.

Vainio, S. and Lin, Y. (2002) Coordinating early kidney development: lessons from gene targeting. Nat Rev Genet 3: 533–543.

Vega, Q.C., Worby, C.A., Lechner, M.S., Dixon, J.E. and Dressler, G.R. (1996) Glial cell line-derived neurotrophic factor activates the receptor tyrosine kinase RET and promotes kidney morphogenesis. Proc Natl Acad Sci USA 93:10657–10661.

Watanabe, T. and Costantini, F. (2004) Real-time analysis of ureteric bud branching morphogenesis in vitro. Dev Biol 271: 98–108.

Winnier, G., Blessing, M., Labosky, P.A. and Hogan, B.L. (1995) Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. Genes Dev 9:2105–2116.

Wong, A., Bogni, S., Kotka, P., de Graaff, E., D'Agati, V., Costantini, F. and Pachnis, V. (2005) Phosphotyrosine 1062 is critical for the in vivo activity of the Ret9 receptor tyrosine kinase isoform. Mol Cell Biol 25:9661–9673.

Xu, X., Weinstein, M., Li, C., Naski, M., Cohen, R.I., Ornitz, D.M., Leder, P. and Deng, C. (1998) Fibroblast growth factor receptor 2 (FGFR2)-mediated reciprocal regulation loop between FGF8 and FGF10 is essential for limb induction. Development 125:753–765.

Yamamoto, M., Cui, L., Johkura, K., Asanuma, K., Okouchi, Y., Ogiwara, N. and Sasaki, K. (2006) Branching ducts similar to mesonephric ducts or ureteric buds in teratomas originating from mouse embryonic stem cells. Am J Physiol Renal Physiol 290:F52–F60.

Young, H.M., Hearn, C.J., Farlie, P.G., Canty, A.J., Thomas, P.Q. and Newgreen, D.F. (2001) GDNF is a chemoattractant for enteric neural cells. Dev Biol 229:503–516.

Yu, H.M., Liu, B., Chiu, S.Y., Costantini, F. and Hsu, W. (2005) Development of a unique system for spatiotemporal and lineage-specific gene expression in mice. Proc Natl Acad Sci USA 102:8615–8620.

Yu, J., McMahon, A.P. and Valerius, M.T. (2004) Recent genetic studies of mouse kidney development. Curr Opin Genet Dev 14:550–557.

Zhang, H. and Bradley, A. (1996) Mice deficient for BMP2 are nonviable and have defects in amnion/chorion and cardiac development. Development 122:2977–2986.

Zhang, X., Ibrahimi, O.A., Olsen, S.K., Umemori, H., Mohammadi, M. and Ornitz, D.M. (2006) Receptor specificity of the fibroblast growth factor family, part II. J Biol Chem 281:15694–15700.

Zhao, H., Kegg, H., Grady, S., Truong, H.T., Robinson, M.L., Baum, M. and Bates, C.M. (2004) Role of fibroblast growth factor receptors 1 and 2 in the ureteric bud. Dev Biol 276: 403–415.