Cell Biology of Ureter Development

Adrian S. Woolf* and Jamie A. Davies†

*School of Biomedicine, University of Manchester, Manchester Academic Health Science Centre and Manchester Children’s Hospital, Manchester, United Kingdom; and †Centre for Integrative Physiology, Hugh Robson Building, University of Edinburgh, Edinburgh, United Kingdom

ABSTRACT

The mammalian ureter contains two main cell types: a multilayered water-tight epithelium called the urothelium, surrounded by smooth muscle layers that, by generating proximal to distal peristaltic waves, pump urine from the renal pelvis toward the urinary bladder. Here, we review the cellular mechanisms involved in the development of these tissues, and the molecules that control the process. We consider the relevance of these biologic findings for understanding the pathogenesis of human ureter malformations.


Molecule Abbreviation Box

ALK Activin receptor-like kinase (growth factor receptor)
AngII Angiotensin II (growth factor)
BMP Bone morphogenetic protein (growth factor)
DLGH Discs-large homolog (intracellular scaffolding protein)
ERK Extracellular signal-regulated kinase (intracellular signaling molecule)
ETV ETS transcription factor (transcription factor)
FGFR Fibroblast growth factor receptor (growth factor receptor)
FOX Forkhead box (transcription factor)
FRAS Fraser syndrome (basement membrane molecule)
FREM FRAS1-related extracellular matrix (basement membrane molecule)
GDNF Glial cell line-derived neurotrophic factor (growth factor)
GATA GATA-binding factor (transcription factor)
GFR GDNF family receptor (growth factor receptor)
HCN3 Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 3 (ion channel)
HNF1B Hepatocyte nuclear factor 1B (transcription factor)
KIT v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (growth factor receptor)
MYOCD Myocardin (transcription factor associated protein)
PAX Paired box (transcription factor)
PI3K Phosphatidylinositol 3-kinase (intracellular signaling molecule)
PLC Phospholipase C (intracellular signaling molecule)
PTCH Patched (growth factor receptor)
RET Rearranged during transfection (growth factor receptor)
ROBO Roundabout (growth factor receptor)
ROCK Rho-associated protein kinase (intracellular signaling molecule)
SMAD Homologs of Drosophila protein, mothers against decapentaplegic and Caenorhabditis elegans protein SMA (intracellular signaling molecule)
SHH Sonic hedgehog (growth factor)
SLIT Slit homolog (growth factor)
SOX SRY-related HMG-box (transcription factor)
TBX T-box (transcription factor)
TGF Transforming growth factor (growth factor)
TSHZ Teashirt (transcription factor)
UPK Uroplakin (urothelial membrane protein)
VANGL Van Gogh-like (planar cell polarity protein)

The nephric (or Wolffian) ducts (NDs) are a pair of epithelial tubes, each of which runs along the edge of the intermediate mesoderm near the body cavity. Each ND gives rise to a ureteric precursor, the ureteric bud (UB), which grows into metanephric mesenchymal (MM) cells condensing out of intermediate mesoderm. Normally, a single bud emerges from each ND near its distal (caudal) end, a precision facilitating optimal interaction between the UB and MM, which are required to generate a single ureter-kidney functional unit of normal shape and internal structure.1–3

In principle, normal budding could be controlled either by prepatterning within the duct itself or by external signals. Experiments with explanted NDs provide no evidence for a strong intrinsic prepatterning. Instead, any part of the duct, even the more proximal (cranial) section lying alongside the mesonephric kidney, can be stimulated to emit ectopic UBs by applying select molecules,4–8 the actions of which are understood by considering intracellular pathways under their control7 (Figure 1).

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Correspondence: Dr. Adrian S. Woolf, School of Biomedicine, University of Manchester, Manchester Academic Health Science Centre and Manchester Children’s Hospital, Michael Smith Building, Oxford Road, Manchester, M13 9PT, UK, or Dr. Jamie A. Davies, Centre for Integrative Physiology, Hugh Robson Building, 15 George Square, Edinburgh EH8 9XD, UK. Email: adrian.woolf@manchester.ac.uk or jamie.davies@ed.ac.uk

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UB emergence is antagonized by SMAD signaling but favored by ERK, PI3K, and PLC activation. NDs express activin A, which acts in an autocrine manner to activate SMADs and prevent budding. However, when an isolated ND is treated with both an activin antagonist and a growth factor that activates ERK, PI3K, and PLC pathways, multiple buds emerge along its length. In such experiments, numerous normal diameter buds rather than one large cyst are generated, implying a yet-to-be-defined lateral inhibition mechanism whereby bud tip cells direct their immediate neighbors to remain quiescent.

ND cells express various cell surface receptors, each of which binds pro-branching or antibranching factors. RET and FGFR2 receptor tyrosine kinases, and their GFRα and sulfated glycosaminoglycan coreceptors, bind GDNF and FGFs, activating ERK, PI3K, and PLC pathways driving UB emergence. Expression of such receptors depends on duct cells expressing the GATA3 transcription factor, and β-catenin, a multifunctional intracellular protein, and on nearby stromal cells synthesizing retinoic acid, an effector metabolite of vitamin A. The extent of intracellular signaling triggered by receptor tyrosine kinases is limited by the cytoplasmic protein sprouty-1, without which the ND produces multiple ectopic buds. In addition, signaling between SLIT2 and ROBO2, members of molecular families first implicated in neural guidance, together with expression of FOXC1 transcription factor, guard against UB ectopia by limiting the cranial extent of the GDNF expression domain within intermediate mesoderm.

As alluded to above, bud emergence is also antagonized by TGFβ family members (activins and BMPs), autocrine and paracrine factors that bind ALK receptor threonine kinases, activating the SMAD pathway. Normally, in vivo, SMAD activation is favored along most of the ND. By contrast, near the duct’s caudal end, MM secretes the BMP antagonist, Gremlin-1 (GREM1), and the RET agonist, GDNF; these, together with AngII-mediated Sprouty-1 (SPRY1) downregulation, favor formation of a solitary, correctly placed UB (Figure 2). An autocrine loop involving neuromodulator Y may enhance the commitment of these ND cells to budding.

ND budding is preceded by increased epithelial proliferation and thickening to a pseudostratified epithelium. RET signaling leads to rearrangement of ND cells such that those with the greatest ERK/PI3K/PLC activation move together and produce the bud. This movement is also modulated by ETV4 and ETV5, transcription factors upregulated by GDNF/RET signaling. During UB branching, epithelial cells become wedge shaped, implicating cytoskeletal changes involving actin microfilaments. Indeed, mutation of genes encoding for the actin depolymerizing factors, coflin 1 and destrin, affect

**Figure 1.** Intracellular pathways modulating UB emergence from the ND. Pathways that encourage (green) and pathways that inhibit (red) bud emergence are depicted. (See also the Molecule Abbreviation Box.)

**Figure 2.** Growth factors controlling UB emergence from the ND. The caudal part of the embryo, with the cloaca/urogenital sinus, is on the left of the diagram. Except near the MM, inhibitory signals such as BMP4 and activin dominate the molecular landscape. MM produces activators such as GDNF in addition to GREM1 and follistatin, which respectively antagonize the anti-branching factors BMP4 and activin. At this precise point, the balance between activation and inhibition favors emergence of a single UB. (See also the Molecule Abbreviation Box.) Note that mesonephric tubules are not formed by ND branching but arise from adjacent mesenchyme.
branching, as does inhibition of ROCK, a molecule driving actin rearrangements. ROCK is itself modulated by the planar cell polarity protein, VANGL2. In vitro, UB epithelia undergo apoptotic death if physically separated from MM, and mesenchymal-derived factors such as GDNF may facilitate UB survival as well as emergence. The PAX2 transcription factor is normally expressed in the ND and the emerging bud and is antiapoptotic in the UB/collecting duct lineage. Prominent ND/UB apoptosis and impaired UB formation occurs in embryos lacking HNF1B (also known as vHNF). This transcription factor is normally expressed in the ND/UB, where it may directly upregulate PAX2.

**WHAT HAPPENS TO THE TOP AND BOTTOM OF THE BUD?**

Once the UB enters the MM, it begins to branch to produce kidney collecting ducts. Consideration of these events is beyond the remit of this review and has been covered elsewhere. It is unclear how similar the mechanisms of UB emergence are to its subsequent arborization. Interestingly, the proximal-distal axis of the UB does not initially restrict the branching ability of its cells because, experimentally, a collecting duct tree can be generated from either end of the nascent ureter.

The just-formed ureter is separated from the urogenital sinus, the bladder precursor, by a length of ND extending beyond the point of UB emergence. When development is complete, however, the ureter connects directly to the bladder, an anatomic change requiring substantial remodeling. Previous teaching postulated that the caudal-most ND cells migrated into the base of the bladder, where they formed the urothelium of the trigone, the triangular zone between the ureteric orifices and the urethral outlet of the bladder; as this occurs, the ureter/ND junction would approximate to the bladder wall. Lineage tracing of genetically labeled ND cells shows that the first part of this model is incorrect. In fact, the caudal-most part of the ND involutes by apoptosis induced by signals from the forming bladder. The vesicoureteric junction then becomes physically separated from the opening of the ND, maintained in males as the ejaculatory duct, as they are pushed apart by growth of the bladder wall.

**FURTHER GROWTH AND DIFFERENTIATION OF URETERIC EPITHELIA**

The shaft of the UB, between the kidney and the ND, grows and differentiates to become the mature ureter. In contrast to UB initiation, less is known about the cell biology of ureteric growth. Once emerged, the bud runs straight to the MM but the guidance mechanisms are not understood. When extra UBs are induced with beads soaked in stimulatory growth factors, they do not always grow toward the beads, arguing against simple chemotaxis. Initial extension of the emerging UB depends on its epithelium expressing FRAS1. This basement membrane protein acts in a complex with two related molecules, FREM1 and FREM2, probably optimizing presentation of MM-derived growth factors to the bud and also physically stabilizing UB/MM interactions by binding integrin α8. A similar lack of UB progression occurs in mutant mice lacking this matrix receptor, which is normally expressed on the surfaces of MM cells.

As it extends, the bud becomes thinner than the zone of ND that produced it, suggesting cell rearrangements involving convergent extension, which is known to drive the remarkable longitudinal growth of Malpighian kidney tubules in fly embryos. Ureters are shorter than normal in TBX18 null mutant mice. This transcription factor is normally expressed in mesenchymal cells surrounding the urethral stalk and its absence is associated with decreased epithelial proliferation. Once initiated, further longitudinal growth occurs in isolated wild-type embryonic ureters maintained in organ culture and in ureters of certain mutant embryos lacking kidneys. Both observations show that exposure to fetal urine is not needed for longitudinal growth, although these experiments do not rule out a more subtle, differentiation-optimizing influence conferred by urine flow that, in mice, probably begins several days after UB initiation when the metanephros has formed its first layers of vascularized glomeruli (Figure 3).

Urothelia in both the ureter and bladder have evolved to stop movement of urine back into the body. Prevention of movement of water and solutes through the apical-most epithelial layer is mediated by plaques made of UPK protein heterodimers. UPK expression occurs early in urinary tract development, being present in epithelia lining the urogenital sinus. In mutant mice lacking either UPK3A or UPK2 proteins, plaques are disorganized and urothelia are leaky. These animals also have malformed urinary tracts with gaping (instead of normal slit-like) vesicoureteric junctions, and dilated ureters associated with either reflux of urine from the bladder or occlusion by exuberant urothelial growth. These structural anomalies might simply be secondary disruptions following on from loss of the urothelial physical barrier. It has, however, been postulated that they may also result from perturbed intracellular signaling by analogy with the proven role for uroplakin proteins in triggering embryogenesis in frog eggs. UPK expression is compromised in ureters of mouse embryos engineered to have
downregulated BMP4 or TBX18, both proteins being normally expressed in adjacent SM precursor cells. Furthermore, application of BMP4 to explanted metanephroi induces UPK expression in ureteric bud branch tips within the organ, suggesting that these UB descendants can be reprogrammed into a urothelial fate.

**URETERIC MUSCLE FORMATION AND FUNCTION**

The shaft of the embryonic ureter initially comprises an epithelial tube extending through loose mesenchyme. This epithelium acts as a paracrine signaling center, driving surrounding cells to differentiate into smooth muscle (SM). The urothelium secretes SHH, a growth factor that binds to the PTCH1 receptor in immediately adjacent mesenchymal cells, stimulating them to proliferate. Peri-urothelial mesenchymal cells are also stimulated to express BMP4, which itself effects their own differentiation into SM. Here, BMP4 enhances in immediately adjacent mesenchymal factor that binds to the PTCH1 receptor and synergizes with the SHH signal through a transcription factor–like protein. TSHZ3 is needed for MYOCD expression within nascent ureteric SM cells. MYOCD, a transcriptional coactivator, then upregulates genes coding for muscle contractile proteins, such as smooth muscle actin and myosin heavy chains. Lack of another transcription factor, SOX9, which like TSHZ3, is normally expressed by mesenchyme aggregating around the urothelial ureteric tube, also leads to failed SM differentiation.

The aggregation of SM precursor cells around urothelia depends on mesenchymal expression of TBX18 and, in mice engineered to lack this transcription factor, prospective SM precursors become mislocalized to the surface of the metanephros. Correct orientation of ureteric SM cells depends on DLGH1, an intracellular scaffolding protein highly expressed in urothelia and more weakly in nascent SM cells. When DLGH1 is inactivated, circular muscle bundles misalign in a longitudinal orientation. In mutant embryos lacking this protein, the differentiation of stromal cells between the urothelium and SM layer is perturbed, suggesting that stroma may somehow control SM bundle alignment. Cell lineage experiments shown that ureteric SM is distinct from muscle layers in the wall of the urinary bladder. After the shaft of the ureter has become enveloped with SM, there appears to be a secondary wave of muscle differentiation at the proximal end (top) of the ureter where it merges into the renal pelvis. These events are mediated by the protein phosphatase, calcineurin, and by AngII signaling.

Mice genetically engineered to lack key molecules in the ureteric SM–differentiation pathway have the common phenotype of hydronephrosis. This arises not from anatomic obstruction but because of a back-up of urine in a functionally obstructed tube lacking normal peristaltic waves.

Forming a network within the SM layers are neural-like, KIT receptor tyrosine kinase expressing cells that are required for generation of contraction waves beginning before birth. Notably, the explanted fetal ureter, even when physically disconnected from the kidney and bladder, undergoes regular peristalsis in a proximal to distal direction. In vivo, peristalsis is triggered by HCN3, a hyperpolarization-activated cation channel expressed in the renal pelvis/kidney junction. When hedgehog signaling is downregulated experimentally in this region, expression of KIT and HCN3 are compromised and contractions are perturbed, even though SM cells themselves appear intact. The mature ureter also contains adrenergic, cholinergic, nitrogic, and sensory nerves, the activities of which modify its contractility.

A theoretical scheme, in which the onset of fetal urine production by the kidney enhances ureteric SM differentiation and function, is depicted in Figure 3.

**IMPLICATIONS FOR UNDERSTANDING HUMAN CONGENITAL URETER MALFORMATIONS**

The human ureter is affected by several types of malformation. The most severe, and rarest (about 0.01%–0.1% births), is its unilateral or bilateral absence, characteristically accompanied by kidney agenesis. Ureteric dilation associated with ureteropelvic junction obstruction or primary megaureter affects up to 0.2% births (reviewed by Lye et al.). Even more common is ureteric duplication (2% of the population); in its most severe form the kidney is also “duplex,” with the top part connected to an obstructed ureter with an ectopic termination in the urethra or vas deferens, and the bottom part connected to a refluxing ureter that inserts too laterally in the bladder wall. Vesicoureteric reflux affects at least 0.5% of births, with some estimates of incidence an order of magnitude higher. Sometimes these malformations occur as part of a syndrome affecting other parts of the body, whereas at other times, the renal lesions occur in isolation.

Knowledge of how specific molecules control ureteral development helps us understand why mutations of certain genes cause human disease. Fraser syndrome often features bilateral ureter and renal agenesis and can be caused by biallelic mutations of either FRAS1 or FREM2, each encoding a UB basement membrane protein. Furthermore, mutations of RET are implicated in humans with similarly severe renal tract malformations. Mutations in ROBO2 are reported in individuals born with refluxing and/or duplicated ureters. Congenitally dilated ureters can occur in humans who have mutations of SOX9 (in Campomelic dysplasia) or GLI3 (Pallister–Hall syndrome), encoding a transcription factor involved in SHH signaling. By analogy with the mouse models described earlier, one may postulate that such ureters may be obstructed functionally because of undifferentiated and/or poorly functioning MS bundles.

In the uro-facial syndrome features vesicoureteric reflux and dysfunctional urinary tract contractility. These individuals have mutations of HPSE2, which codes for heparanase-2, a protein of similar structure to classic heparanase. Both genes are expressed in the fetal ureter and bladder, where they are
postulated to regulate neuromuscular functional differentiation. **UPK3A** mutations have been reported in humans born with ureteric malformations\(^\text{47}\) resembling those described in mice genetically engineered to lack the encoded urethelial plaque protein. Genes encoding for several other proteins (PAX2, GATA3, HNF1B) implicated in ureter development are mutated in human renal tract malformations.\(^\text{72–74}\) In some instances, the gene in question is also normally expressed in the kidney and will have intrinsic roles in this organ as well as in the ureter. Accordingly, the manifest renal malformation may reflect multiple primary aberrations of upper and lower tract development. Here, a good example is HNF1B, where human mutations cause both ureteric atresia and cystic dysplastic kidneys.\(^\text{74}\) Another is PAX2, where human mutations associate with both vesicoureteric reflux and kidney hypoplasia.\(^\text{73}\)

### Table 1. Transcripts with strong and specific ureteric expression in developing mice, as reported in the GUDMAP database

<table>
<thead>
<tr>
<th>Transcript</th>
<th>Description</th>
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<tbody>
<tr>
<td>Esrb</td>
<td>(estrogen-related receptor β)</td>
</tr>
<tr>
<td>Hnf4g</td>
<td>(hepatocyte nuclear factor 4 γ)</td>
</tr>
<tr>
<td>Hoxa1</td>
<td>(Homeobox 1α)</td>
</tr>
<tr>
<td>Isl1</td>
<td>(Islet 1)</td>
</tr>
<tr>
<td>Lhx6</td>
<td>(Lim Homeobox gene 6)</td>
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<td>Lix1</td>
<td>(Lim expression 1)</td>
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<tr>
<td>Mdb1</td>
<td>(Myelin basic protein)</td>
</tr>
<tr>
<td>Neurod4</td>
<td>(Neurogenic differentiation 4)</td>
</tr>
<tr>
<td>Nrap</td>
<td>(Nebulin-related anchoring protein)</td>
</tr>
<tr>
<td>Tox3</td>
<td>(Tox high mobility group box family member 3)</td>
</tr>
<tr>
<td>Zfhx4</td>
<td>(Zinc finger homeobox 4)</td>
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</tbody>
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**GUDMAP, GenitoUrinary Development Molecular Anatomy Project.**

**ON GOING DISCOVERY OF NOVEL OR UNSUSPECTED URETER DEVELOPMENT GENES**

The genetic search for new human ureteric malformation genes continues, with numerous loci suggested by genome-wide analyses.\(^\text{75–77}\) Fortunately for human geneticists, and also those studying the basic mechanisms of renal tract development, there is open access to a resource that makes high-throughput analyses of gene expression freely available to all. The GenitoUrinary Development Molecular Anatomy Project\(^\text{78}\) database holds information on RNA array analyses from microdissected tissues in the developing murine urogenital system.\(^\text{79}\) At the time of writing, there are also >1450 in situ hybridization entries showing gene expression in the developing ureter. Examples of transcripts that have a particularly strong and specific ureteric expression are shown in Table 1. Cross-referencing with the Online Mendelian Inheritance in Man database,\(^\text{67}\) to ascertain whether any have been associated with human disease and/or might fit into what is already known about the biology of ureter development, revealed the following points. **HOXA1** mutation is associated with a brainstem dysgenesis syndrome, although the state of the renal tract was not reported; **ISL1** is a known activator of BMP4 expression; **MXN1/HLXB9** mutations are implicated in the Curradino syndrome, characterized by anorectal and sacral malformations and which can sometimes feature duplex ureter, hydroureter, vesicoureteric reflux; and **Nrap** encodes a protein implicated in anchoring of myofibrillar proteins.

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