How Does the Ureteric Bud Branch?

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ABSTRACT
Many genes that modulate kidney development have been identified; however, the molecular interactions that direct arborization of the ureteric bud (UB) remain incompletely understood. This article discusses how “systems” approaches may shed light on the structure of the gene network during UB branching morphogenesis and the mechanisms involved in the formation of a branched collecting system from a straight epithelial tube in the context of a stage model. In vitro and genetic studies suggest that the stages seem to be governed by a conserved network of genes that establish a “tip-stalk generator”; these genes sustain iterative UB branching tubulogenesis through minimal alterations in the network architecture as a budding system shifts to one that autocatalytically branches through budding. The differential expression of stage-specific positive and inhibitory factors in the mesenchyme, likely presented in the context of heparan sulfate proteoglycans, and effector molecules in the epithelium seems to regulate advancement between stages; similar principles may apply to other branching epithelia such as the lung, salivary gland, pancreas, mammary gland, and prostate. Active mesenchymal interactions with the UB seem to govern vectorial arborization and tapering of the collecting system and its terminal differentiation. Cessation of branching correlates with induction of mesenchyme as well as local extracellular matrix changes. Perturbations of these mechanisms and/or single-nucleotide polymorphisms in genes regulating UB branching may predispose to a variety of renal diseases (e.g., hypertension and chronic kidney disease) by altering nephron number. Decentralization of the gene–protein interaction network may explain the relative paucity of branching phenotypes in mutant mice and in human disease.


Ureteric bud (UB) branching morphogenesis is fundamental to establishing the architecture of the kidney and is a key determinant of nephron number. This process is important not only for normal renal function but also from the standpoint of disease. Although it is clear that kidney malformations such as renal agenesis and dysplasia are caused by defective morphogenesis of the UB, emerging data suggest that the predisposition to several common diseases such as hypertension and chronic kidney disease have similar developmental origins.

The renal architecture primarily arises through the growth and morphogenesis of two progenitor tissues, the UB and metanephric mesenchyme (MM). Through a process of mutual induction between these tissues, the UB is formed through an outpouching of the Wolffian duct (WD) and undergoes a number of iterative dichotomous branching events to form the urinary collecting system while the MM is induced to undergo a mesenchymal-to-epithelial transformation to form the nephron, from the epithelial glomerulus to the distal tubule (Figure 1A). A comprehensive discussion regarding the many factors that have been discovered to modulate UB branching and mesenchymal-to-epithelial transition is beyond the scope of this article and has been the subject of excellent reviews.1–3 Here we discuss the general principles that lead to the formation of a branched ureteric tree; such principles may apply to other branching epithelia as well.

Creation of Tips and Stalks

In vitro and genetic approaches have helped to identify many promoters and inhibitors of UB branching (reviewed in1,2), but the fundamental mechanism of how a straight epithelial tube gives rise to a branched tree remains obscure. Before the advent of systems specifically designed to study the UB, branching morphogenesis of the UB was described through the analysis of branching in renal cell lines such as Madin-Darby canine kidney (MDCK) and UB cells4–6; however, recent data suggest that UB branching proceeds through a fundamentally different mechanism of outpouching of wedge-shaped cells that are created through an apical cytoskeletal “purse-string” mechanism (Figure 2A).7
The “branching through budding” model implies that the remodeling of the contiguous epithelial tube occurs through the creation of a secretory epithelium via differential localization of growth factor receptors and matrix-degrading enzymes to UB tips relative to stalks that initiate new branch formation, a concept supported by cell lineage studies, and microarray analysis of differential gene expression in UB tip versus stalk cells. Thus, this challenges the traditional notion that the epithelial tube is composed of homogeneous cells and suggests that microenvironments within the UB, possibly in the form of gradients, are key to vectorial branching morphogenesis and the generation of new tips.

**KIDNEY DEVELOPMENT IS A STAGED PROCESS**

In addition to the creation of tips and stalks, branching morphogenesis underpins the basic architecture of the kidney. Global patterns of gene expression during kidney organogenesis, together with in vitro and genetic data and morphologic analyses, suggest that branching morphogenesis of the UB is an iterative yet simultaneously vectorial process that can be broadly conceptualized in terms of developmental stages: (1) Outgrowth of the UB from the WD; (2) rapid, iterative branching of the UB; (3) deceleration of UB branching accompanied by differentiation of the mesenchymal mesenchyme; and (4) termination of branching and completion of mesenchymal differentiation (Figure 1A). These stages are separable into in vitro modules that have been used to reconstitute “engineered” kidney tissue that is capable of early vascularization and rudimentary tubular function. Each stage is typified by various sets of heparin-binding growth factors, receptor tyrosine kinases, signaling pathways involving intracellular kinases, and effectors that mediate cell adhesion and basement membrane remodeling (reviewed in2,16–18). Although disruption of certain pathways produces catastrophic effects (i.e., renal agenesis), numerous instances in which mutation...
of key molecules has minimal apparent phenotypic consequences exist, suggesting that each of these stages is characterized by a distinct network structure of gene and protein interactions that confer varying resilience to mutation.19,24,25

**UB OUTGROWTH**

The initiating step in metanephric development is emergence of the UB from the WD; failure of this critical step leads to renal agenesis, whereas incorrect positioning of the UB leads to a variety of urinary tract anomalies ranging from mega-ureter to vesicoureteral reflux. A multitude of positive and negative regulatory factors, converging on glial cell–derived neurotrophic factor (GDNF) signaling, play key roles in this stage (reviewed in16). Genetic deletion of GDNF or its receptor, Ret, most often results in renal agenesis, although rudimentary kidneys form in up to 50% of these mice,20–22 suggesting that GDNF-dependent budding of the WD may be bypassed through activation of other signaling pathways. This concept has been validated through in vitro studies in which the combined effect of stimulatory (fibroblast growth factor 7 [FGF7]) and blockade of inhibitory (activin) molecules is able to induce bud formation in isolated WDs.23 The existence of such a bypass pathway may be sufficient to explain the relative infrequency of renal agenesis despite the seeming dependence of UB outgrowth on GDNF signaling; therefore, it may be that a “second hit” is necessary to manifest the phenotype.

**EARLY UB BRANCHING**

The next conceptualized stage of collecting system development, rapid iterative UB branching, depends on reciprocal epithelial–mesenchymal interactions. A common theme emerging from the study of diverse branching systems is the existence of both positive and negative feedback loops in the complex interplay among epithelium and mesenchyme.24 Like UB outgrowth (and subsequent stages), a combination of stimulatory (e.g., FGFs, pleiotrophin, heregulin) and inhibitory (e.g., TGF-β1, bone morphogenetic protein 4, activin) molecules have been shown to modulate the extent of ureteric branching, but, in general, positive feedback mechanisms seem to predominate.14

The iterative, or “feed-forward,” nature of the branching tree suggests that the expression of a particular set of proteins becomes stable and self-maintaining such that an autocatalytic network, or “tip-stalk generator,” is established (Figure 1A)22; therefore, only a minimal alteration in the expression of a small set of genes may be required for the impressive morphologic changes that occur between the stages of UB branching. Such a “consensus set” of conserved signaling molecules can also be found among a number of organs, and it is the relative “weight” of certain pathways (along with those guiding final differentiation) that may be organ specific.24 Thus, it may be the structure of the branching network through the capture of nodes and the “tightness” of the links between nodes that determines epithelial cell fate.25 Such self-organization has been proposed in gene regulatory networks in origin of life scenarios.27

The question remains, then, how is the complex patterning of the nephron achieved? To explain patterning during embryogenesis, the concept of gradient morphogens was proposed more than a century ago.28 Secreted proteins of the WNT; hedgehog; and members of the EGF, FGF, and TGF-β families, all of which have been found to be important in kidney morphogenesis, have been recognized as candidate substances to provide positional information (reviewed in12,29,30). That TGF-β may be an arbiter of tubule spacing has been explored in other branching systems in which TGF-β repulses epithelial cell processes to space out the branches of the epithelial tree.12,31,32 It is interesting to note that most of the secreted proteins involved in UB branching are heparin binding. Heparan sulfate proteoglycans are known to modulate cell surface localization of ligands and thus are ideal candidates as positive and negative regulators of signaling by morphogen gradients (Figure 1B).33 In this context, heparan sulfate proteoglycan diversity has been proposed to be a key (although largely unexplored) driving mechanism of stage-specific regulation of UB morphogenesis.34

**LATE UB BRANCHING**

As organogenesis comes to completion, branching slows down, presumably owing to negative feedback. In the kidney and other branching systems, it seems that members of the TGF-β superfamily are the primary molecules involved in branching inhibition, but there is mounting evidence that negative feedback signals may also arise from mesenchyme cell surface molecules.3,14 This is anatomically suggested in the kidney as fusion between a lateral ureteric branch, and metanephric tubule effectively removes the ureteric branch from further divisions; however, signals to slow branching may be present even earlier during MM-derived tubule formation, where it has been noted that branch-inhibitory factors are expressed in comma and S-shaped bodies and thus may regulate the extent and pattern of branching (reviewed in26).

Mutations in the pathways that regulate early and late UB branching usually do not result in significant branching defects.11 The phenotypes that are manifest are generally quantitative leading to a decrease in nephron number. There is evidence that low nephron number in humans predisposes to hypertension and chronic kidney disease35,36; although it is unlikely that essential hypertension is due to a loss-of-function mutation in a single gene, it is conceivable that variant alleles, identified by single-nucleotide polymorphisms, that interact differently with modifiers, suppressors, and enhancers are sufficient to cause subtle changes in nephron number that eventually lead to disease.11

**BRANCHING CESSION**

The events that signal the termination of branching remain enigmatic. Miscues in
the cessation of kidney development can lead to a variety of renal disorders ranging from reduced nephron number to cystic kidney disease, which can be considered a disorder of tubule maintenance.\textsuperscript{11} In vitro studies suggest soluble factors produced by the differentiating MM modulate the expression of specific subsets of matrix proteases that can modify the extracellular matrix and influence branch termination.\textsuperscript{12} For example, release of endostatin at UB tips modulates UB branching and specific sizes and concentrations of the basement membrane component hyaluronic acid seems to independently regulate UB branching and promote tubular maturation.\textsuperscript{37,38} Thus, extracellular matrix components may act as a potential switch for ending branching morphogenesis, as well as initiating nephron differentiation.

Stop/mutation signals also seem to be correlated with the differentiation of the MM.\textsuperscript{39} UB-derived soluble factors that promote mesenchymal epithelialization, such as leukemia inhibitory factor, inhibits branching of the isolated UB.\textsuperscript{14,40} In mice, loss of nephrogenic mesenchyme (as a result of conversion into nephrons) at the time of birth leads to the disruption of dichotomous branching module. It seems likely that the signaling network, resulting in a branching module. It seems likely that this branching module is conserved among multiple branching organs,\textsuperscript{26} and although it seems plausible to hypothesize a unifying theory of epithelial branching through the establishment of an autocatalytic network, there are undoubtedly pathways that are stage and organ specific. It will be through elucidation of those pathways that we will understand what makes a kidney a kidney and why perturbation of specific pathways results in renal disease.

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**DISCLOSURES**

None.

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