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Making a Tubule the Noncanonical Way

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Tubulogenesis is an essential feature during formation of multiple organ systems. The genitourinary, cardiovascular, pulmonary,...
auditory, pulmonary, and gastrointestinal systems all require the formation of epithelialized tubes for proper function. Interest in the developmental mechanisms controlling this process is heightened by the relationships with disease-inducing processes. In the case of the kidney, tubule morphogenesis can inform us about the etiology of cystic kidney diseases and other pathologic conditions, while the isolation of disease-associated genes has greatly contributed to the elucidation of developmental mechanisms. In this issue of JASN, Miller et al. demonstrate that planar cell polarity (PCP) components of the Wnt pathway are expressed in the developing pronephric kidney and are necessary for tubule morphogenesis.

Noncanonical Wnt signaling, a term referring to signals not involving the canonical effector, β-catenin, is broadly but not precisely synonymous with the PCP pathway. The latter, as originally defined in Drosophila, controls polarity of individual cells and within sheets of cells in epithelia, linking the pathway firmly to cilia, often the most obvious manifestation of cell polarity. The PCP pathway, in turn, proves essential for morphogenetic cell movements in vertebrates, notably, the movements that shape the embryo during gastrulation. In this process cells converge toward the midline and the embryo extends along the anterior-posterior axis, yielding the term convergent extension for these movements.

Generally when one thinks about Wnt-mediated PCP signaling and kidney organogenesis, thoughts invariably drift toward cilia formation and function. The connection between PCP signaling and ciliopathies arises from the fact that they share multiple essential genes. For example, PCP genes such as Vangl2, Fritz, and Inversin/NPHP2 play a role in ciliogenesis, while genes disrupted in ciliopathies such as Bardet-Biedl Syndrome (BBS) are required for convergent extension in Xenopus and zebrafish embryos. Yet a bona fide connection between cilia and Wnt signaling remains tenuous, in contrast to the solidly established relationship between cilia and Hedgehog signaling.

There is no question that Wnt signaling plays a key role during kidney development, but how the canonical and noncanonical branches of the Wnt pathway fit into the kidney organogenes is picture has not been thoroughly elucidated. Multiple ligands stimulate the canonical or noncanonical branches, which themselves may be subdivided into PCP and Ca\(^{++}\)-dependent pathways. Various ligands are known to preferentially, but not exclusively, activate one or the other branch of the pathway, depending on cell type and developmental context. During kidney development, the earliest, and still most extensive, evidence implicates canonical Wnt signaling as a key factor in nephron induction. While many Wnt ligands are expressed in and adjacent to the developing kidney—and several have been shown to be involved in this process—Wnt4 and Wnt9b have been most extensively studied and shown, by genetic deletion studies in the mouse, to be essential for kidney organogenesis. Wnt4 has also been shown to have an essential role in kidney tubulogenesis by antisense morpholino oligonucleotide (MO) studies in Xenopus. Likewise, gene ablation experiments demonstrated that β-catenin, the effector of the canonical pathway, is necessary for specification of the embryonic renal progenitor cell field. However, forced β-catenin expression, while initiating kidney induction, does not lead to proper nephrogenesis, suggesting that the canonical pathway needs downregulation during later stages of the development of this organ.

At the same time, evidence has been accumulating for a role of noncanonical Wnt signaling in nephrogenesis. Indeed, some recent evidence indicates that only noncanonical Wnt signaling, without any involvement of β-catenin activation, regulates tubulogenesis in metanephric mesenchyme and controls renal cyst formation in inversin mutant mice, a model of nephronophthisis type II. Therefore, it has become paramount to elucidate the particular branches within the noncanonical pathway that control specific stages of kidney organogenesis and to identify the downstream mediators of those signal transduction cascades. This approach is exemplified by two recent studies.

Tanigawa et al. used metanephric mesenchyme cell culture to show, convincingly, that Wnt4 induces renal tubules without the stabilization of β-catenin, and with complete insensitivity to inhibition of the canonical Wnt pathway. The authors further show the relevant branch of the noncanonical pathway in this system involves Ca\(^{++}\) influx, leading to the phosphorylation of CaMKII. It appears that phosphorylated CaMKII can terminate canonical Wnt signaling, which is necessary during later stages of kidney organogenesis because, as noted above, continued Wnt/β-catenin activity interferes with the tubule differentiation process.

The current study in JASN by Miller et al. uses a different model system, the Xenopus embryo, to study noncanonical Wnt signaling during kidney development. Xenopus laevis studies have the advantage that it is possible to inhibit the expression of certain molecules in specific lineages by injecting MOs into a particular blastomere. This is essential when studying the Wnt/PCP pathway, as general inhibition of this pathway interferes with convergent extension movements. Using this approach, the authors ask whether two known signaling components of the Rho GTPase branch of the Wnt PCP pathway are required for the formation of pronephric kidney tubules. The formin family protein, Daam1, and the GTP Exchange Factor, WGEF, are required for the transduction of Wnt signaling in the PCP pathway through the small GTPase Rho, and Daam1 function links to rearrangement of the cytoskeleton. The authors find that both factors are expressed in the pronephric kidney field and are essential for tubulogenesis. While the involvement of Daam1 and WGEF is consistent with other work implicating PCP pathway components in this process, the depletion of these two factors does not lead to cystic kidneys in Xenopus, whereas disruption of several
other PCP components does.\textsuperscript{12,13} This distinction may point to additional branching of the pathway or to temporal or cell type-specific requirements for subsets of pathway components during kidney organogenesis.

Notwithstanding the progress summarized above, it is clear we still lack a comprehensive understanding of the molecular and cellular mechanisms driving kidney organogenesis. Determining that a signaling pathway, or certain components of the pathway, are necessary for kidney development is a good first step, but ultimately we need to appreciate the detailed regulatory interactions that occur during organogenesis, and how these interactions affect the behavior of cells from the specification of progenitor populations to the terminal differentiation and spatial arrangement of the cell populations that form the functional organ. Such knowledge will provide a solid basis for the interpretation of disease processes in which particular developmental pathways have gone awry, impairing organ function with often-catastrophic consequences for the entire organism.

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DISCLOSURES

None.

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See related article, “Pronephric Tubulogenesis Requires Daam1-Mediated Planar Cell Polarity Signaling,” on pages 1654–1667.