REVIEW ARTICLE

Kidney Development Branches Out

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ABSTRACT   For more than 40 years now, the developing kidney has served as a model paradigm for epithelial-mesenchymal interactions. The principles of inductive signaling, epithelial cell differentiation, and pattern formation are now being addressed with modern genetic and biochemical tools. In addition to the mammalian kidney organ culture model, both zebrafish and *Xenopus laevis* demonstrate great potential for investigating the molecular mechanisms of kidney organogenesis within a whole organism. In this review, the papers presented in this special issue are discussed with respect to recent progress in the renal development field. Coincidentally, it has become increasingly clear that progress made in renal development can impact our understanding of the genetic basis of disease. Dev. Genet. 24:189–193, 1999.   © 1999 Wiley-Liss, Inc.

Key words: kidney development; pronephros; *Xenopus*; zebrafish

INTRODUCTION

In simplest terms, developmental biologists concern themselves with a relatively small number of basic principles that can be applied to most any complex system, be it a multicellular organism or a specific tissue within an organism. Much of the conceptual frameworks that model embryonic development evolved to explain how pluripotent cells give rise to many specialized, terminally differentiated cells, by repeated cell division and increasing restriction of potency, and how positional information is translated in time and space such that the three-dimensional architecture of the embryo is realized. Although cell lineage analysis reveals the developmental potential of a precursor cell, transplantation experiments to ectopic positions are often necessary to determine when the fate of a precursor cell becomes restricted. With the advent of gene cloning and transgenic technologies, the molecular pathways underlying the specification of position and cell fate are becoming clearer and reveal striking conservation among genetic networks in diverse systems.

In mammals, the kidney, or metanephros, is induced at a time when much of the body plan is already established, all three axes are clearly defined, and most of the major internal organs can be discerned as rudimentary tissues with a few simple precursor cell types. Yet, the process of organogenesis has only just begun, as each major tissue type undergoes a unique program of differentiation. Much of the classical work in the developing kidney has focused on the signals and the consequences of induction, as defined by the contact of the ureteric bud epithelium with the metanephric mesenchyme. These experiments are thoroughly reviewed in Saxen's [1987] classic monograph, with several more current reviews [Davies and Bard, 1998; Lechner and Dressler, 1997] discussing the progress made since that time. The purpose of this short review is not to provide extensive background but rather to introduce the papers in this special issue of *Developmental Genetics* and put them into a larger context.

The molecular basis of renal development is now being addressed in many species, each with unique advantages. Furthermore, renal developmental biology has provided new insights toward understanding clinical disease mechanisms and may ultimately point the way toward novel therapies. Thus, it is wholly justifiable to cover the broad spectrum of renal development in a variety of species and to correlate these with human disease, given the conserved genetic mechanisms that may ultimately underlie both processes.

EMERGING MODEL SYSTEMS

Since the pioneering work of Grobstein [1956], the kidney has long been recognized as an attractive model

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for epithelial-mesenchymal inductive interactions. Trans-filter organ culture systems were developed to address the inductive signaling events between the two primordial tissues, the ureteric bud and the metanephric mesenchyme. However, it should be stressed that the inductive signals that promote aggregation of the mesenchyme and epithelial conversion are primarily permissive. The mesenchyme, an aggregate of posterior intermediate mesodermal cells, will only make the renal epithelium and cannot be instructed otherwise. The question remains then as to what specifies this metanephric mesenchyme within the posterior intermediate mesoderm. The answers are not readily amenable in mammalian systems, although gene knockout experiments point to several candidate transcription factors. Several labs have begun to explore the *Xenopus* pronephros as a model for early kidney development [for review, see Vize *et al.*, 1997]. Given the evolutionary relationship, the conservation of gene expression patterns, and the apparent common cell lineages, it seems probable that a similar mechanism could specify the pronephric anlagen of the intermediate mesoderm in *Xenopus* and the metanephric mesenchyme in the posterior intermediate mesoderm of mammals. Indeed, the expression patterns of the *Xenopus* homologues of *WT1*, *Pax2*, *Lim1,* and *Wnt4*, all key regulators of mammalian metanephric development, partition the intermediate mesoderm into the different components of the pronephros prior to formation of any epithelial structures [see Carroll *et al.*, 1999, in this issue]. Given the potential for dominant gain of function phenotypes in the *Xenopus* embryo, it will be important to determine which of these factors, if any, can extend the boundaries of, or transform, one pronephric region into another. The function and evolution of multigene families expressed in the kidney can also be addressed in lower vertebrates by examining expression patterns of closely related genes. For example, the *Pax2*, *Pax5*, and *Pax8* genes are probably derived from a single ancestral gene yet their expression patterns show subtle but significant differences between *Xenopus* and mouse [Heller and Brandli, 1999, in this issue]. Thus, *cis*-acting regulatory sequences of the individual genes may have evolved separately in these species to differentially control gene expression in the intermediate mesoderm, thymus, and otic vesicle.

The second important new model organism for the study of organogenesis in general is the zebrafish. Large-scale genetic screens have proven the utility of zebrafish embryos for the identification of new loci required for the correct development of the heart, the nervous system, the gastrointestinal system, and the pronephric kidney [Hafter *et al.*, 1996; Driever *et al.*, 1996]. More recently, 15 complementation groups have been described that affect zebrafish pronephric development, the majority of which result in rapid glomerular and/or tubular cyst formation [Drummond *et al.*, 1998]. Although the transparency of the zebrafish embryo allows for the identification of mutants in the pronephros, the presence of cysts may be a clear phenotype that is easily scored whereas cellular transformations or more subtle tubular defects might go undetected. In the prototype cystic mutant *double bubble*, epithelial cell polarity and transport, as well as the glomerular basement membrane, appeared disturbed suggesting an essential function for this gene in glomerulogenesis. Such mutants can be used to revisit old issues in the renal development field. In this issue, the relationship between endothelial cells and glomerular podocytes is addressed using the zebrafish mutant *cloche*, which essentially ablates all endothelial cells at an early stage [Majumdar and Drummond, 1999]. Although organ culture experiments suggested that podocytes could differentiate in the absence of discernible endothelial cells, the in vivo analysis in the *cloche* mutant clearly underscores this point. Other pronephric mutations identified in zebrafish include the homologue of *Pax2/5*, named *no ismuth* (*noi*) because of the hindbrain defects, which like its mammalian counterparts is required for tubular epithelium formation along the entire nephric duct [Brand *et al.*, 1996]. It remains to be seen how many novel genes will be discovered through the mapping and cloning of such zebrafish mutations. Clearly the potential for additional screens using more sensitive and targeted phenotypic analysis for pronephric development should generate sufficient excitement to make the zebrafish a valuable model within the renal community.

EARLY METANEPHRIC DEVELOPMENT

Regarding the origin of the epithelial cells of the kidney, the prevailing view dictated that the ureteric bud, an outgrowth of the nephric duct, induces the metanephric mesenchyme to convert to an epithelium and generate most, if not all, of the epithelial cells of the nephron. Reciprocally, the mesenchyme induces the ureteric bud to proliferate and branch, thus generating the collecting duct system. This classical view was based primarily on morphological analysis and it was not until the lineage tracing studies of Qiao *et al.* [1995] that this model was challenged. By marking individual cells in the mesenchyme or ureteric bud and following their development in organ culture, it became clear that the developmental potential of either tissue is not as restricted as previously thought. Ureteric bud cells could delaminate and contribute to epithelial cells in the proximal tubules, just as mesenchyme cells could be found in more distal structures, even presumed collecting duct epithelium. These experiments were technically demanding and were not readily accepted. As reported in this issue, Arend *et al.* [1999] utilized a different strategy to arrive at essentially the same results. By making chimeric kidney rudiments using mouse urteric bud and rat mesenchyme, or vice versa, and doing species-specific reverse-transcription poly-

merase chain reaction (RT-PCR) for markers known to localize only in specific epithelial subtypes, the authors show that much of the nephron appears to be derived from both mesenchyme and ureteric bud, with the most distal tubules coming predominantly from the bud. Lineage tracing and ureteric bud branching morphogenesis can now be studied with a new transgenic mouse that expresses green fluorescent protein (GFP) in the ureteric bud epithelium [Srinivas *et al.*, 1999, in this issue]. Live images of cultured kidneys can now be monitored to see more clearly if epithelial cells delaminate from the bud. Hopefully GFP will be stable enough to follow such cells for at least a short time if they down regulate the HOX promoter used to drive expression.

What triggers the metanephric mesenchyme to undergo epithelial conversion? This question has remained at the forefront of renal developmental biology for close to 40 years. The transfilter induction assay, with heterologous inducers such as the spinal cord, has been used extensively to characterize the properties of inductive signals. It appears now that WNT proteins mimic the induction activity associated with the spinal cord [Kispert *et al.*, 1998]. In particular, WNT4-expressing cells can promote tubule formation when in contact with mesenchyme explants, dissected free of ureteric bud. One caveat of the transfilter assay is that the mesenchyme is separated from the ureteric bud and may already have been exposed to some primary, bud-derived signal. Indeed, PAX2 expression can already be discerned in mesenchymal cells near the bud even before the first branch point is evident. Thus, WNT4 may provide a secondary signal that propagates or amplifies some primary inductive event. In vivo, *Wnt4* mutants also show *Pax2* expression in the mesenchyme but arrest development at the aggregate stage [Stark *et al.*, 1994]. Thus, it seems likely that some initial signal, derived from the bud, is required for *Pax2* activation and subsequent WNT4 signaling, derived from the aggregating mesenchyme, is required for epithelial conversion. Whether *Pax2* activation affects the wnt signaling pathways in the mesenchyme directly or predisposes the cells to respond to wnt signals remains to be determined.

The inductive signals that promote urteric bud branching have received much attention over the last 3 years. Clearly, the RET receptor [Schuchardt *et al.*, 1994] and its complexed ligands, GDNF [Moore *et al.*, 1996; Pichel *et al.*, 1996] and GFRα1 [Enomoto *et al.*, 1998], are key components of a signaling pathway that stimulate bud outgrowth and branching morphogenesis. Within the context of the posterior nephric cord, GDNF is sufficient to induce ectopic ureteric bud outgrowth [Sainio *et al.*, 1997] and most likely does so by acting as a chemoattractant for RET-expressing epithelial cells [Tang *et al.*, 1998]. The tip of the ureteric bud expresses several unique markers, with respect to the more mature epithelium of the stalk, such as WNT11, RET, and c-ros. Persistent expression of wnt11 appears

to depend on activated RET, as it is abolished upon inhibition of RET signaling [Ehrenfels *et al.*, 1999, in this issue] and increases upon RET stimulation by exogenous GDNF [Pepicelli *et al.*, 1997]. Inhibition of RET signaling also results in mesenchymal changes that may reflect the decrease in wnt11 emanating from the tip of the bud. Surprisingly, though, wnt11 is one of only two wnt proteins tested that do not mimic the induction activity associated with the spinal cord in the classical mesenchyme induction assay [Kispert *et al.*, 1998]. The GDNF-related protein neurturin can also signal through the RET kinase through the GPI-linked receptor, GFRα2 [Klein *et al.*, 1997; Buj-Bello *et al.*, 1997]. Like GDNF, neurturin can promote ureteric bud branching and distention in vitro [Davies *et al.*, 1999, in this issue]. Yet, its expression in ureteric bud epithelium suggests an autocrine mechanism of action. However, the cellular localization of neurturin and GFRα2 in the developing kidney must be determined more precisely to clarify this hypothesis.

How does the mesenchyme respond to inductive signals and generate epithelium? From the early aggregate stage to the formation of s-shaped bodies and finally mature glomeruli and tubules, the changes in mesenchyme morphology are profound. Changes in gene expression patterns accompany every step of this sequential process. Yet which proteins are the driving forces behind cell movement, differentiation, and proliferation of the primitive epithelial cells? Cell adhesion proteins of the cadherin family may promote aggregation and compartmentalize the developing nephron into distinct regions along the developing tubules [Cho *et al.*, 1998]. Expression of new integrins and extracellular matrix components can also change the shape of the primitive epithelial structures. Defining the precise spatial and temporal sequence of gene expression, from the induced mesenchyme to the mature nephron, is a necessary, albeit Herculean, task that must be completed before a complete understanding of these morphogenetic processes will be at hand. The renal community is fortunate that much of the available gene expression data has been kept up to date in an easily accessible database that can now be subject to more advanced search strategies [Davies, 1999, in this issue]. Despite the number of entries in the database, the earliest changes in gene expression upon induction of the mesenchyme remain obscure. The paper by Leimeister *et al.* [1999] in this issue begins to address this problem by reporting a well-controlled screen for genes activated in the early induced mesenchyme. By using in vitro induced mesenchyme and differential display, genes activated after 1, 2, or 3 days postinduction could be identified. Clearly, this approach is only the beginning and suffers from the same problem of distinction between initial bud-derived vs. propagating spinal cord-derived signals. However, such large-scale screens will surely provide new information and identify critical genes whose expression may be limited in time and space.

RENAL DEVELOPMENT AND DISEASE

A distinct advantage of studying development in an organ system such as the kidney is that the regulatory mechanisms may provide insight into the pathogenesis of human renal disorders. Indeed, Wilms' tumor, polycystic kidney disease (PKD), and renal carcinoma are all thought to encompasses elements of developmental pathways gone awry. The *WT1* gene is an early regulator of metanephric development whose loss of function is associated with a low percentage of human Wilms' tumors. Strikingly, *Wt1* mouse mutants have no kidneys because the metanephric mesenchyme is unable to respond to inductive signals and undergoes apoptosis [Kreidberg *et al.*, 1993]. However, the *Wt1* mutant mesenchyme does appear morphologically distinct and expresses markers such as GDNF [Donovan *et al.*, 1999, in this issue]. Yet, *Wt1* mutant mesenchyme expresses *Pax2* RNA but not protein. In light of recent reports localizing WT1 isoforms to the splicing machinery [Davies *et al.*, 1998], it is worth asking whether *PAX2* mRNA splicing is regulated, positively or negatively, by WT1.

The mouse *Wt1* mutant phenotype is clearly different than what is observed in humans, where *WT1* null cells, arising by a second hit or the loss of heterozygosity, are within the environment of a normal developing kidney. Heterozygous mice do not undergo a second hit with nearly the same frequency as humans. In order to model Wilms' tumor in the mouse, it may be necessary to transplant *Wt1* null mesenchyme cells into a normal developing newborn kidney. Transplantation of mutant tissue into a wildtype host can take the analysis of mutant mice to the next level. Such an approach was taken by Rogers *et al.* [1999, in this issue] to show the effects of IGFI depletion in a postnatal kidney. Although *Igf1* null mice appeared to have normal kidneys at birth, the newborns die shortly thereafter and the kidneys were not amenable to physiological analysis. By transplanting *Igf1* mutant kidneys into rats, the authors demonstrate a role for IGF1 in determining nephron number and in maintaining renal physiology.

The identification of two genes associated with human PKD, *PKD1* and *PKD2*, has now enabled investigators to study the developmental role of these genes and to generate mouse models for autosomal dominant PKD. Although the *PKD1*-associated disease is inherited in a dominant manner, initiation of cystic disease occurs late in life and appears to require multiple second hits, much like a tumor suppressor gene, to generate clonal populations of cysts [Qian *et al.*, 1996]. Mice homozygous for a *Pkd1* loss-of-function allele acquire cysts very early, even before birth [Lu *et al.*, 1997], whereas humans generally have a late onset. Thus, the mouse model highlights an essential function of PKD1 during epithelial cell differentiation, whereas the human disease demonstrates that epithelial cells can revert to a less differentiated phenotype in the absence of a maintenance function. Possible roles for the PKD1 protein, polycystin, include specification of cell polarity, binding to the extracellular matrix, or cell-cell communication. Given the size of polycystin and the multitude of domains present in the protein, it will be difficult to dissect the function without an experimental model system. Working under the assumption that the PKD repeats found in the extracellular domain may bind an unknown ligand, van Adelsberg [1999, in this issue] utilized peptides of these repeats, as competitive inhibitors of PKD1 function, in the kidney organ culture model. The data suggest a role for polycystin in the branching morphogenesis of the ureteric bud. Yet in the absence of more specific information regarding extracellular binding proteins for polycystin, it is hard to reconcile these effects with the disease phenotype.

Progress in PKD research has been aided by the availability of several well-characterized mouse models. Although the gene has not been identified, the congenital polycystic kidney (*cpk*) mouse shares many features with human PKD, including mislocalization of transmembrane proteins and changes in basement membrane composition. Using the *cpk* mouse model, Gattone *et al.* [1999, in this issue] demonstrate that the inability of *cpk* kidneys to concentrate urine is not due to lack of expression of appropriate genes. Rather, it may be due to physical distention such that the thick ascending limb cannot establish a gradient. Strikingly, inhibition of the argenine vasopressin V2 receptor reduces cyst growth, suggesting that cAMP is an integral part of the cystogenic pathway. Although much work needs to be done, such studies begin to address the physiological basis of fluid secretion and the state of differentiation in the cystic epithelial cell.

SUMMARY

The field of kidney development is diversifying into new and potentially more amenable systems. While continuing to focus on the classic problems of induction and epithelial conversion, many of the signaling molecules and transcription factors regulating early development are at hand and the intimate link between development and disease is being realized. Ultimately, the genetic pathways that direct pluripotent mesenchymal cells to make renal epithelium may become clear. It would not be within the realm of science fiction to imagine one day replicating such genetic pathways in vitro to engineer a viable kidney for replacement therapy. We can dream, can't we?

ACKNOWLEDGMENTS

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