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What is needed for kidney differentiation and how do we find it?

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What is needed?

The permanent vertebrate kidney, the metanephros, like many other complex tissues and organs, comprises several cell lineages. These include the mesenchymal metanephric blastema, the epithelial anlage bulging from the Wolffian duct, the vasculature including the highly specialized glomerular structures, and the neuronal component. A prerequisite for normal development of the kidney with its numerous organized nephrons is a strict control of the differentiation and spatial assembly of the four cell lineages and their derivatives. This is achieved by an intimate communication, a morphogenetic or inductive interaction, between the cells throughout development. Both theoretically and, to some extent experimentally, the complex problem can be dissected into a series of interactive events, many of which have been successfully explored by various strategies (review, Saxén, 1987).

The mesenchymal blastema

It is a derivative of the caudal end of the nephrogenic cord. Following an epithelial conversion it gives rise to the secretory part of the nephron consisting of the glomerular epithelium as well as the proximal and distal tubules, ultimately joining the epithelium-derived collecting duct system (Fig. 1). For both theoretical and experimental purposes, it is important to emphasize that the mesenchymal blastema, at the onset of overt morphogenesis, is not an uncommitted, "virgin" target to be modified by guiding forces. Tissue recombination experiments have have shown that, in fact, the cells have already undergone predetermination and developed a tubule-forming, bias as no other embryonic cells at this stage can be experimentally converted into kidney-specific structures (Saxén, 1970). Moreover, the blastema seems to consist of several stem-cell lineages with different developmental options (Bard *et al.,* 1996, and below).

The epithelial component

It initially buds from the caudal Wolffian duct, invades the mesenchymal blastema, and undergoes there a dichotomous branching (Fig. 2). After fusion to secretory nephrons it constitutes the collecting system of the kidney. The spatially and temporally strictly synchronized development of the mesenchyme-derived structures and the collecting duct epithelium suggests a guiding interaction between them. This reciprocal morphogenetic interaction has been convincingly demonstrated in separation and recombination experiments, indicating that the epithelial conversion of the mesenchyme is triggered by signals emitted by the ureter epithelium, and, conversely, that the initial budding and the regular branching of the ureter-derived epithelium are regulated by messages from the mesenchyme (Grobstein, 1955).

The molecular basis of these apparently sequential interactions has been recently elucidated by several authors (Sariola and Sainio, 1997; Vainio and Muller, 1997; this issue).

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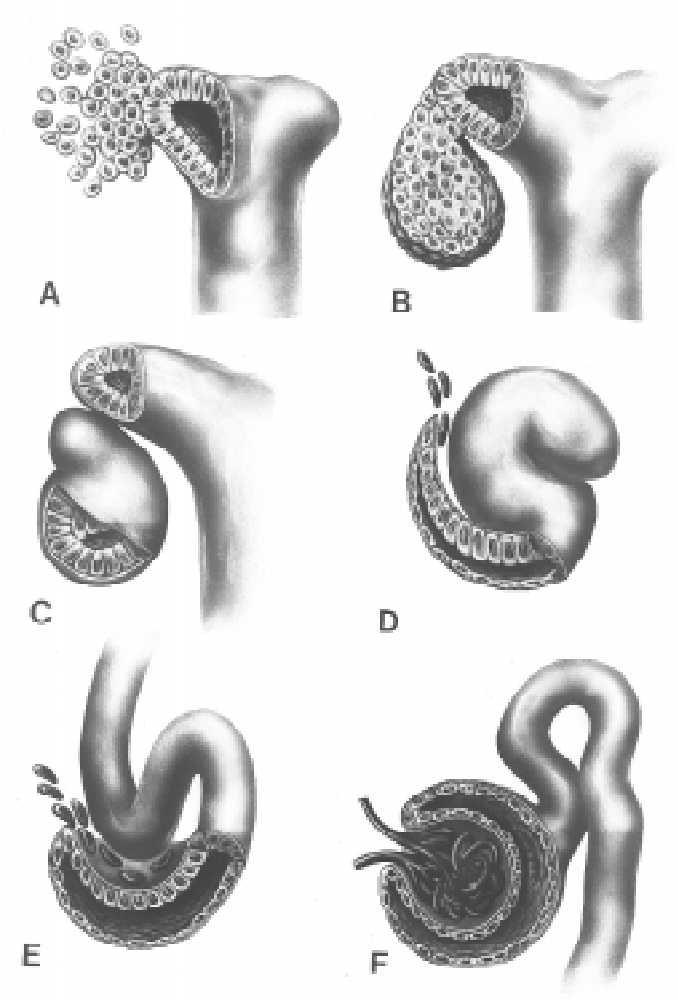

Fig. 1. Semi-schematic illustration of the early development of the nephron from a mesenchymal condensate to the S-shaped body and the glomerulus. *(From Saxén, 1987).*

The vasculature

The vasculature of the kidney can be either of external origin (angiogenesis) or formed *in situ* from stem cells in the multipotent mesenchymal blastema (vasculogenesis). Both modes of development have their adherents with experimental evidence. Grafting experiments using young, avascular nephric blastemas on avian chorioallantoic membrane have demonstrated that capillaries from the host can invade the mesenchyme and contribute to chimeric glomerular structures (Ekblom *et al.,* 1982). This mode of vascular growth would require a precise pathfinding mechanism by which the endothelial cells are guided into the glomerular loop. An interaction between the invading capillary endothelium and the matrix molecules of the mesenchyme has here been postulated (Sariola *et al.,* 1984). These results and conclusions have, however, been recently challenged by Woolf and Loughna (1998), who examined the expression VEGF (vascular endothelial growth factor) which is considered to be "specific" for the endothelial cell lineage. Both VEGFmRNA and the receptors *flk1* and *flt1* were detected in the undifferentiated, avascular metanephric mesenchyme Hence, presently the two alternative modes for kidney vasculogenesis vs angiogenesis should be considered and explored without excluding the possibility of a dual origin of the kidney endothelium *in vivo.*

The neuronal elements

The neuronal elements in the developing kidney have been less explored, but in this case also two origins of the neural component should be considered, an internal and an external one. The former alternative is supported by the observation that an early stromal cell population expresses neuronal markers, such as NF-L and NF-M, whereas the neural cell adhesion molecule L1 is first found in the external microganglia and only subsequently within the blastema (Sainio *et al.,* 1994). Hence, as in vasculogenesis, a double origin of the neural component of the kidney has to be considered.

How do we find it?

An apparent conclusion from the above presentation is that at least four cell lineages which develop in a highly synchronized manner, must be aware of each other´s developmental options to ensure the formation of a strictly organized tissue.

How does a scientist deal with this complex problem? Various strategies have been developed and implemented - many of which are applicable to other models-systems as well.

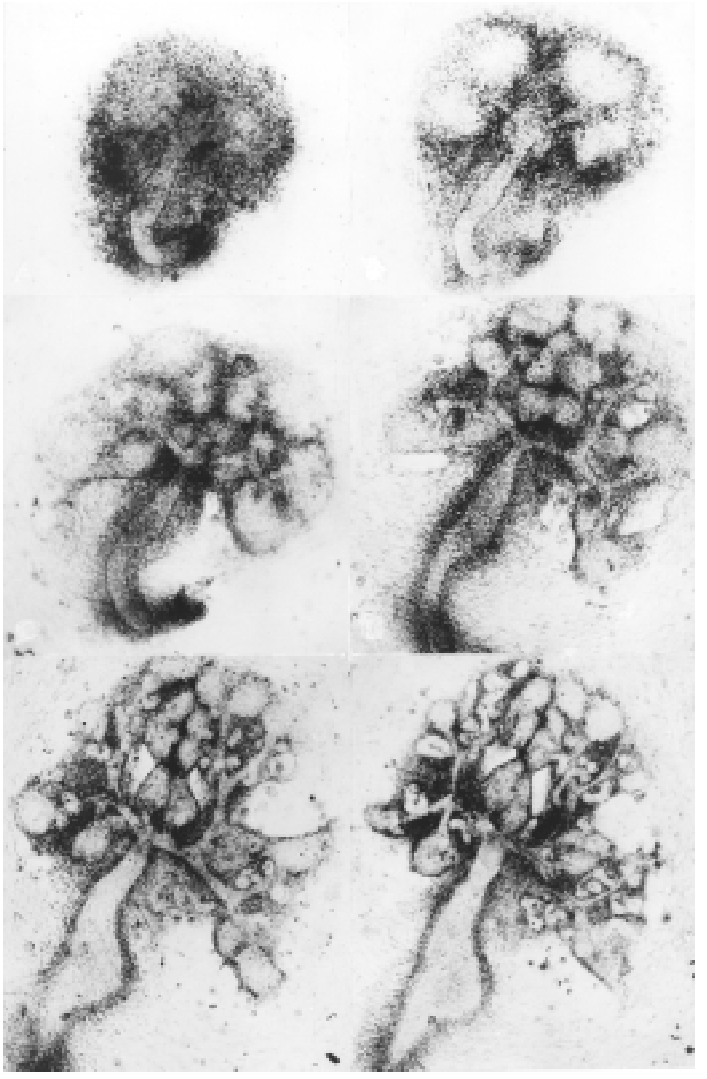

Fig. 2. Daily prints of a time-lapse motion picture of an 11-day mouse metanephric rudiment cultivated *in vitro*. *(From Saxén and Wartiovaara, 1966).*

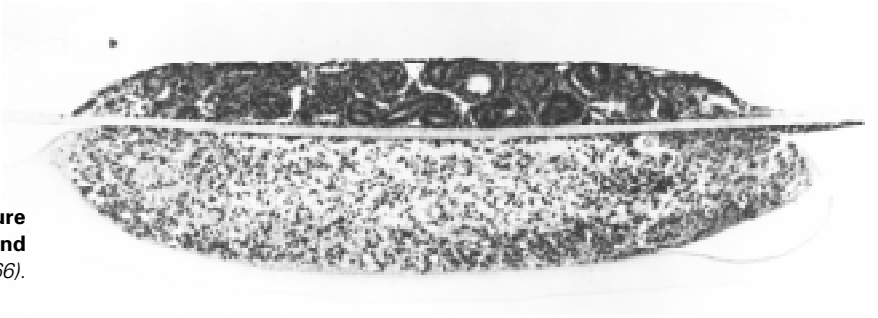

Fig. 3. Micrograph of a section from a transfilter culture set-up showing developing tubules above the filter and inductor tisue (below). *(From Saxén and Wartiovaara, 1966).*

Morphological analyses

Microscopy supplemented with immunohistochemistry and autoradiography for binding assays have yielded important observations. A special advantage here is the mode of kidney development which follows a centrifugal mode of differentiation and maturation of the nephrons, as the inductive ureter gradually invades the responsive mesenchymal blastema. Consequently, a whole series of developmental stages of the nephron can be detected and visualized by various techniques in a single section of the metanephros.

TABLE 1

SOME TRANSGENIC MOUSE STRAINS WITH DEFECTS IN KIDNEY MORPHOGENESIS (FROM THE KIDNEY DEVELOPMENT DATABASE, 1998)

Mutant gene *molecular type*	**Transgenic technique**	**Phenotype**
AP-2 *transcription factor*	knock-out	hypoplastic kidneys
Bcl-2 *inhibitor of apoptosis*	knock-out	polycystic kidneys, excess of apoptosis
BF-2 *transcription factor*	knock-out	large condensates, hypoplastic kidneys
BMP-7 *signaling molecule*	knock-out 1	severe renal hypoplasia
BMP-7 *signaling molecule*	knock-out 2	mild renal phenotype, poor distal tubules
Emx-2 *transcription factor*	knock-out	absent kidneys
GDNF *signaling molecul*e	knock-out	absent kidneys
Integrin α8	knock-out	absent kidneys
Integrin α3	knock-out	collecting duct defect, cystic proximal tubules
Lim-1 *transcription factor*	knock-out	absent kidneys
p53 *cell cycle regulator*	overexpression	hypoplastic kidneys, high apoptosis
p57-Kip2 *cell cycle regulator*	knock-out	dysplastic medulla
Pax-2 *transcription factor*	knock-out	absent kidneys
Pax-2 *transcription factor*	overexpression	dysplastic kidneys
PDGF-B *signaling molecule*	knock-out	absent mesangium
PDGF- receptor	knock-out	absent mesangium
Retinoid acid receptor	knock-out	absent kidneys
Ret *GDNF receptor*	knock-out	hypodysplasia
Transforming growth factor β-2 *signaling molecule*	knock-out	unilateral absence of kidneys, dilated renal pelvis
Wnt-4 *signaling molecule*	knock-out	hypoplasia, condensates, no epithelial differentiation
WT-1 *transcription factor*	knock-out	absent kidneys

Microsurgery

It has been a powerful tool in the hands of technically skilled embryologists since the beginning of this century. As regards the metanephros, the crucial operation involves separation of the two major components, the mesenchyme and the ureter bud.

Separate *in vitro* cultivation or grafting of these cell lineages leads to a standstill in the morphogenesis of both components, while their postoperative recombination *in vitro* triggers both epithelial conversion of the mesenchyme and branching of the ureter-derived epithelium. Combination of the interacting tissues through a thin, membraneous filter as designed by Grobstein (1953, 1956) has proven an excellent strategy (Fig. 3). As the initial interaction of the mesenchyme and its inductor in transfilter position is completed in 24 h, well before overt morphogenesis, the interactants can then be re-separated and analyzed separately for both qualitative and quantitative postinductory events (Saxén and Lehtonen, 1978).

In vitro cultivation

In vitro cultivation of the nephric blastema or its separated components as well as their grafting into heterotopic sites have become routine methods which allow various experimentral interference studies with specific coumpounds such as antibodies, metabolic inhibitors and antisense oligonucleotides. Tests for soluble candidates for signal substances emitting induction or otherwise promoting growth and differentiation of the kidney, can be implemented by adding these compounds into the culture medium at varying intervals and concentrations. An improved strategy consists of soaking tiny heparin or agarose beads with the compound(s) to be tested and inserting the beads into various sites of the blastema.

Microchemistry

Microchemistry for the expression, localization and quantitative variations of various gene products in the kidney follows the overall strategies of cell- and molecular biology of today. A special advantage with the kidney model-system, apart from the centrifugal, gradual mode of differentiation, is offered by the transfilter technique. Here both interacting components can be analyzed separately for incorporation studies and other quantitative measurements. Moreover, the separated, undifferentiated mesenchyme offers an adequate reference tissue for reduction analysis of gene activation during nephrogenesis - either in the whole blastema or in the target mesenchyme subcultivated after sufficient transfilter induction (Lehtonen *et al.,* 1997).

Targeted mutations and other transgenic technologies

Targeted mutations and other transgenic technologies affecting kidney development are presently extensively explored by many

groups (below), and a great number of such transgenic or knockout mouse strains are already available (The Kidney Developmental Database, 1998; Table 1). Of the mutated genes, the following seem, at the moment, most interesting for future research: *WT-1* transcription factor, *GDNF* signaling molecule, *α8β1 integrin* and the *Lim-1* transcription factor (fore details see the following articles in this volume: Davies *et al.*; Dressler and Woolf; Sariola and Saarma; Vainio *et al.*). It should be stressed, however, that so far not a single gene unique for kidney development has been found. Thus, the extremely rapid, sequential gene activation during nephrogenesis and the interaction of these genes leading to kidney-specific features (or their defects) remain our main challenge in the years to come.

KEY WORDS: *metanephric kidney, inductive tissue interactions, trans-filter technique*

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