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# REVIEW

# Model Systems for the Study of Kidney Development: Use of the Pronephros in the Analysis of Organ Induction and Patterning

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**Most vertebrate organs, once formed, continue to perform the function for which they were generated until the death of the organism. The kidney is a notable exception to this rule. Vertebrates, even those that do not undergo metamorphosis, utilize a progression of more complex kidneys as they grow and develop. This is presumably due to the changing conditions to which the organism must respond to retain what Homer Smith referred to as our physiological freedom. To quote, "Recognizing that we have the kind of blood we have because we have the kind of kidneys we have, we must acknowledge that our kidneys constitute the major foundation of our physiological freedom. Only because they work the way they do has it become possible for us to have bones, muscles, glands, and brains. Superficially, it might be said that the function of the kidneys is to make urine; but in a more considered view one can say that the kidneys make the stuff of philosophy itself" ("From Fish to Philosopher," Little, Brown and Co., Boston, 1953). Different kidneys are used to make the stuff of philosophy at different stages of development depending on the age and needs of the organism, rather than the usual approach of simply making embryonic organs larger as the animal grows. Although evolution has provided the higher vertebrates with complex adult kidneys, they continue to utilize simple kidneys in embryogenesis. In lower vertebrates with simple adult kidneys, even more simple versions are used during early developmental stages. In this review the anatomy, development, and gene expression patterns of the embryonic kidney, the pronephros, will be described and compared to the more complex kidney forms. Despite some differences in anatomy, similar developmental pathways seem to be responsible for the induction and the response to induction in both evanescent and permanent kidney forms. Gene expression patterns can, therefore, be added to the morphological and functional data indicating that all forms of the kidney are closely related structures. Given the similarities between the development of simple and complex kidneys, the embryonic kidneys may be an ideal model system in which to investigate the genesis of multicomponent organ systems.**
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## 1. NEPHRON ANATOMY AND FUNCTION

All three kidney forms—the metanephros (last kidney), mesonephros (middle kidney), and pronephros (first kidney)—have a similar functional organization and differ mostly in the spatial organization and numbers and types of nephrons utilized to perform these functions.

The nephron is the basic functional unit of all kidneys, and there are a number of different types of nephrons used in vertebrates. The first type of nephron is found in metanephric and mesonephric kidneys and consists of a glomerulus integrated into the tip of a kidney tubule, the Bowman's capsule (Fig. 1). The Bowman's capsule is vascularized by an arteriole which splits into as many as 50 capillaries. The endothelial cells of these capillaries are not completely contiguous, leaving small holes, or fenestrations, which allow fluids to flow out of the blood vessels. The capillaries are supported by a network of endothelial mesangial cells and pericytes, the glomerular basement membrane, and the

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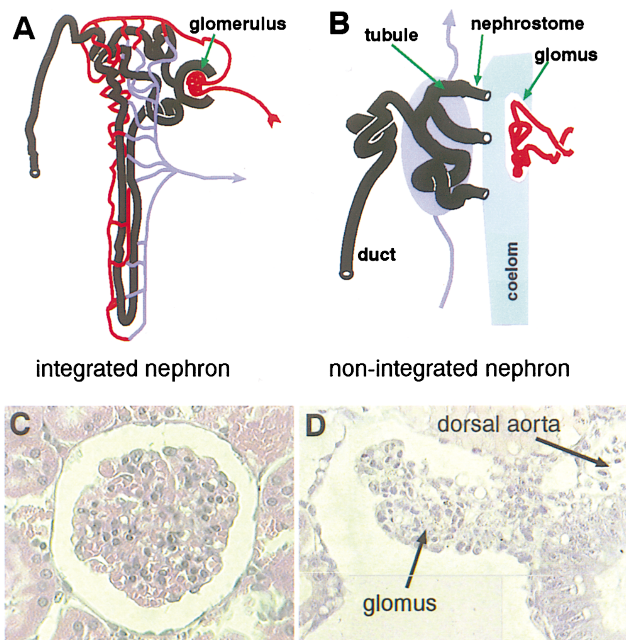


**FIG. 1.** Nephron anatomy. (A) Organization of an integrated nephron. A blood vessel (red) enters the Bowman's capsule where wastes are filtered into Bowman's space. The blood vessel then exits the capsule and surrounds the distal (near the capsule) and proximal tubules. Resorbed nutrients, salts, and water are then returned to the blood stream via the venous system (purple). (B) Organization of a nonintegrated nephron. Wastes filtered into the coelom are collected by the tubules. Resorbed materials are returned to the bloodstream via blood sinus (purple) derived from the posterior cardinal vein that surrounds the tubules. (C) Mammalian glomerulus. (D) Amphibian glomus.

podocytes of the glomerular epithelium. Both the mesangial cells inside the glomerulus and the podocytes surrounding it secrete a basement membrane that surrounds the capillaries and acts as an additional molecular sieve to help separate water and solutes from cells and proteins, the former passing through to the lumen of the Bowman's capsule and the latter remaining in the blood system. The capillaries reunite as they exit the glomerulus, and the first job of the nephron is completed, the ultrafiltration of the blood. The same blood vessel then resplits into fine capillaries that surround the convoluted kidney tubule. Two processes now occur in tandem; the first is the resorption of water, nutrients, and other useful molecules from the filtrate. This is mediated by specific transporter proteins in the kidney tubule epithelia. The transporter proteins will reclaim needed molecules and transfer them to the surrounding capillaries (reviewed by Dantzler, 1988). The second process occurring is tubular excretion. Once again, specific transporters are involved, but in this instance the molecules are being moved from the plasma to the lumen of the kidney tubules. In some organisms glomeruli have been dispensed with entirely (Huot, 1897), and all waste disposal is performed by active tubular excretion mediated by specific transporter proteins (reviewed by Smith, 1951, 1953). The filtrate remaining after tubular resorption and excretion is then disposed of as urine via the Wolffian duct or its derivatives.

Integrated nephrons look very similar in different vertebrate species (Smith, 1953). Minor differences among species include the use of cilia by lower vertebrates in the portion of the tubule adjacent to the glomerulus, the use of the renal–portal system to help collect resorbed materials (in all vertebrates except mammals), and variations in the length and function of the intermediate segment or loop. These latter variations have been reviewed in detail by Dantzler (1988).

The second form of vertebrate nephron is found only in simple embryonic kidneys, the pronephroi. In this instance the vascularized filtration unit is not directly integrated into the kidney tubule. In nonintegrated nephrons, the tubules form on one side of the nephrocoel, and the glomus or glomerulus, the filtration unit, forms on the other. When the vascular structure is one body segment in length it is referred to as a glomerulus, while if it extends over multiple body segments, or contains multiple fused glomeruli, it is referred to as a glomus (plural glomera). The glomus projects into a body cavity known as the nephrocoel. The nephrocoel and the coelom are initially contiguous in both amphibians and fish but later separate into distinct cavities (for a detailed description of the subdivision of the coelom, see Goodrich, 1930).

The organization of a nonintegrated nephron is shown in Fig. 1. In this example, that of a larval amphibian, the dorsal aorta vascularizes a glomus that extends into the coelom. Wastes are filtered from the glomus into the nephrocoel or coelom. Little is known about the fine structure of the glomus, but is it probably very similar to that of the glomerulus, and superficially they look much alike (Fig. 1). Following filtration into the nephrocoel/coelom, fluids are swept into the kidney tubules by thin ciliated funnels referred to as nephrostomes (Figs. 1 and 2). Each dorsal branch of the pronephric tubules has its own nephrostome. Anurans generally have three pronephric tubule branches linked to the coelom by three nephrostomes, most urodeles have two, and teleosts have a single nephrostome joined to an unbranched pronephric tubule. The nephrostomes are thinner than the pronephric tubules and are completely lined with cilia that extend along the entire length of the nephrostome (Fig. 2). Fluids are moved by ciliary action into the pronephric tubules, sometimes referred to as the glandular tubules. The convoluted pronephric tubules are surrounded by a blood sinus into which molecules recovered from the filtrate are returned to the blood stream. Molecules not resorbed in the tubules are disposed of via the Wolffian duct, which is referred to as the pronephric duct when utilized by a pronephros.

Intermediate forms of nephrons are also found, where external glomera or glomeruli become more and more intimately associated with the tubules and the nephrostome is

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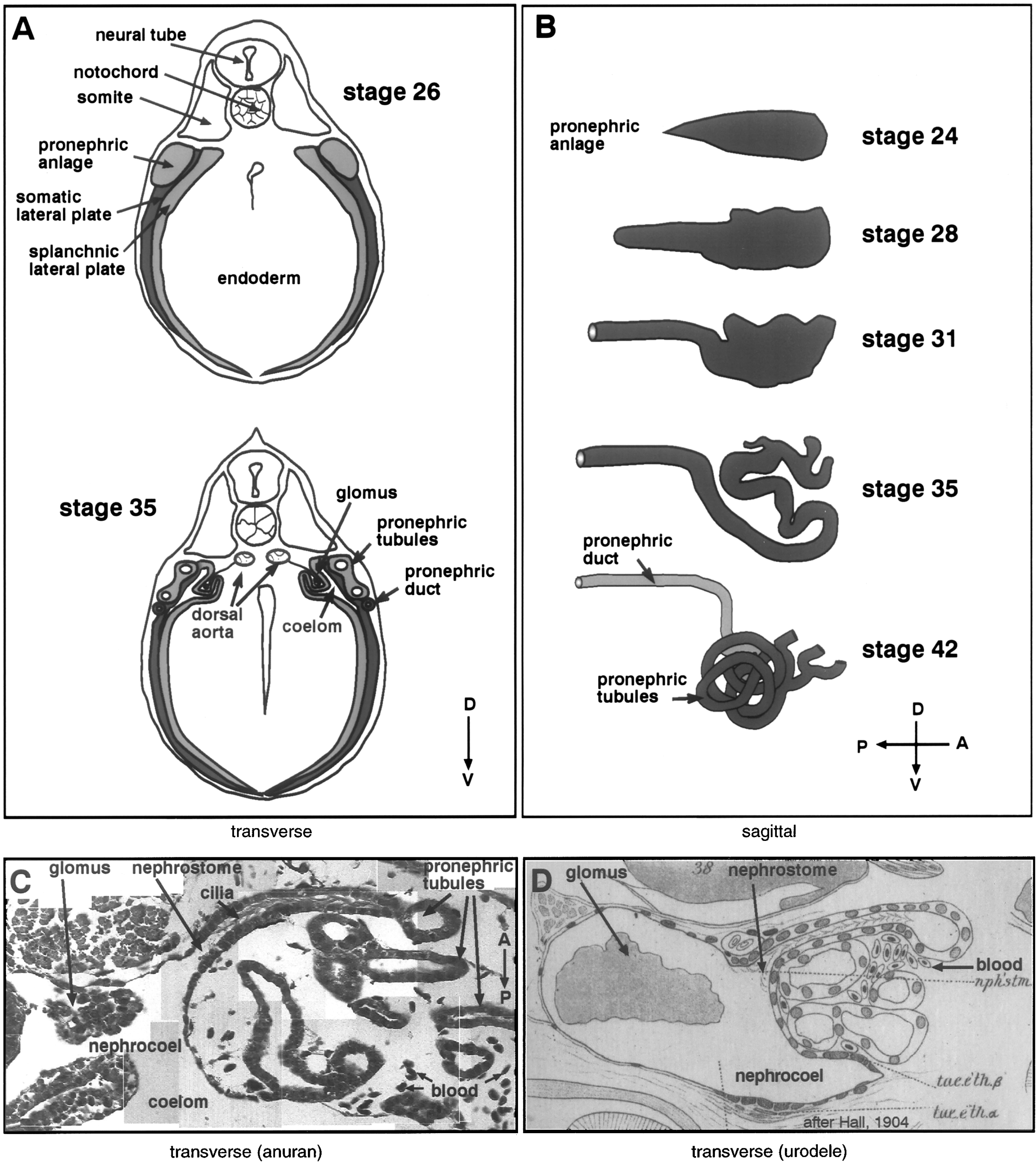


**FIG. 2.** Pronephric development. (A) Development of the *Xenopus* pronephros observed in transverse sections (after Carroll and Vize, 1996). (B) Development of the pronephros observed in sagittal sections (Fields, 1981; Howland, 1921; Vize *et al.*, 1995; Hausen and Riebesell, 1991) from the "tear drop" pronephric anlage of late neurulae to the convoluted pronephric tubules of feeding stages. (C) Shumway (1940) stage 24 *Bombina orientalis* (equivalent to Nieuwkoop and Faber (1994) stage 45) transverse section. Note the ciliated nephrostome linking the broader pronephric tubules to the coelom. (D) Forty-four-millimeter *Ambystoma punctatum* transverse section (after Hall, 1904).

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simply a ciliated portion of the pronephric tubules adjacent to the glomus. This is the situation in teleost pronephroi, where the pronephric cavity (equivalent to the nephrocoel or the glomeral space) into which a central glomus is projected is connected to closely associated pronephric tubules by a ciliated ''neck'' tubule or nephrostome (Marshall and Smith, 1930; Tytler, 1988).

For excellent reviews on the development of integrated nephrons see Saxén (1987) and Clapp and Abrahamson (1994), and for reviews on the development of the glomeruli, see Hyink and Abrahamson (1995). For further details on the development of nonintegrated nephrons, See Section 3.

## 2. ORGANIZATION OF NEPHRONS INTO KIDNEYS

The most complex kidney form is the metanephros, the terminal kidney of reptiles, birds, and mammals (Goodrich, 1930). The metanephros has a branched organization as opposed to the more linear organization of the mesonephros (Fig. 2 and see below). An adult human metanephros contains almost 1 million nephrons (Smith, 1951). Most nephrons are present around the cortex of the organ and the tubule leading from each integrated nephron drains into an ever widening system of collecting ducts (Fig. 3). Most anatomy, morphology or development texts contain descriptions of metanephric anatomy and development. Useful examples include Smith (1951), Saxén, 1987, Dantzler (1988) and Gilbert (1994).

As this form of branched architecture is unique to metanephroi, caution must be used in describing the development of other kidney forms using metanephric terminology. As will be described below, mesonephroi and pronephroi do not form from a ureteric bud and no such structure is present in these organs. Similar cellular interactions, molecules, and signaling pathways are involved (below), but the organization is quite distinct.

The mesonephros is a less complex kidney found in the embryos of higher vertebrates, those that will later develop metanephroi, and in adults of the lower vertebrates. It is also known as the Wolffian body and sometimes in fish and amphibians as the opisthonephros (Kerr, 1919). The term opisthonephroi is used to describe mesonephroi that function as the adult kidney, as opposed to mesonephroi that will later relinquish function to metanephroi. The literal meaning of the prefix opistho- is behind or posterior, and in this context refers to the formation of the mesonephros from the entire posterior portion of the nephrogenic mesoderm rather than reserving the posterior material for a metanephros. Mesonephroi usually contain between 10 and 50 nephrons of two basic types. The first is an integrated nephron, in which a glomerulus is present in a Bowman's capsule, and the second where the mesonephric tubule connects to the coelom via a ciliated nephrostome. Some mesonephric tubules contain both a nephrostomal connection to the coelom and one or more integrated glomeruli (Goodrich, 1930, Nelson, 1953, Balinsky, 1970).

Mesonephric tubules generally form in an anterior to posterior sequence, with the anterior nephrons being formed first and more posterior nephrons being added in a consecutive/sequential fashion (Goodrich, 1930; Nieuwkoop and Faber, 1994). In frogs and fish the earliest mesonephric nephrons often contain nephrostomes, while the latter tubules do not (Nelson, 1953). Mesonephric tubules in mammals and birds do not typically contain nephrostomes (Nelson, 1953). Although mesonephric tubules sometimes connect to the coelom via nephrostomes, they do not have external glomera, and may partially rely on the function of the pronephric glomus or on the diffusion/excretion of wastes into the coelom by the coelomic epithelium. Mesonephroi have a similar vascular system to metanephric nephrons, in which the blood vessel exiting the glomerulus surrounds the tubules to resorb water and nutrients (Fig. 1; Saxén, 1987).

The degree of development of the embryonic kidney in higher vertebrates is correlated to some extent to the development of the placenta. In species where the extraembryonic membranes are intimately associated with the placenta, such as humans and mice, mesonephroi tend to be less well developed compared to species with less effective waste exchange systems, like the pig, which have extensive mesonephroi (Nelson, 1953; Carlson, 1988). The mesonephroi of birds are also well developed (Carslon, 1988). Mesonephroi in lower vertebrates, where it is the adult kidney form, are very well developed with each mesonephric tubule branching a number of times and with each branch containing its own glomerulus.

The most simple form of vertebrate kidney is the embryonic pronephros (Figs. 1–3). In amphibians the pronephros consists of a single glomus filtering wastes into the nephrocoel or coelom, an associated coiled pronephric tubule to collect the filtrate and a duct to dispose of urine. Following filtration into the coelom, fluids are swept into the pronephric tubules by one (fish), two (urodeles), or three (anurans) ciliated nephrostomes. Within the glandular tubules nutrients and water are resorbed. Wastes are transported to the cloaca or urogenital sinus by the pronephric (Wolffian) duct. In essence, the pronephros is one large, nonintegrated nephron.

The organization of pronephroi in teleost fish differs in a few ways from that observed in amphibians. The anatomy of pronephroi has been described for *Fundulas,* toadfish, pipefish, *Microagadus tomcod, Tylosurus marinus* (Armstrong, 1932), mudskipper (Safer *et al.,* 1982), trout (Tytler, 1988), turbot, herring (Tytler, in press), and zebrafish (I. Drummond, personal communication) and is quite similar in all of these species. A glomerulus forms adjacent to the anterior tip of the pronephric tubules in the nephrocoel following the separation of this chamber from the coelom. As development proceeds the two glomeruli migrate toward the midline of the embryo and fuse into a single central glomus that is vascularized by both branches of the dorsal

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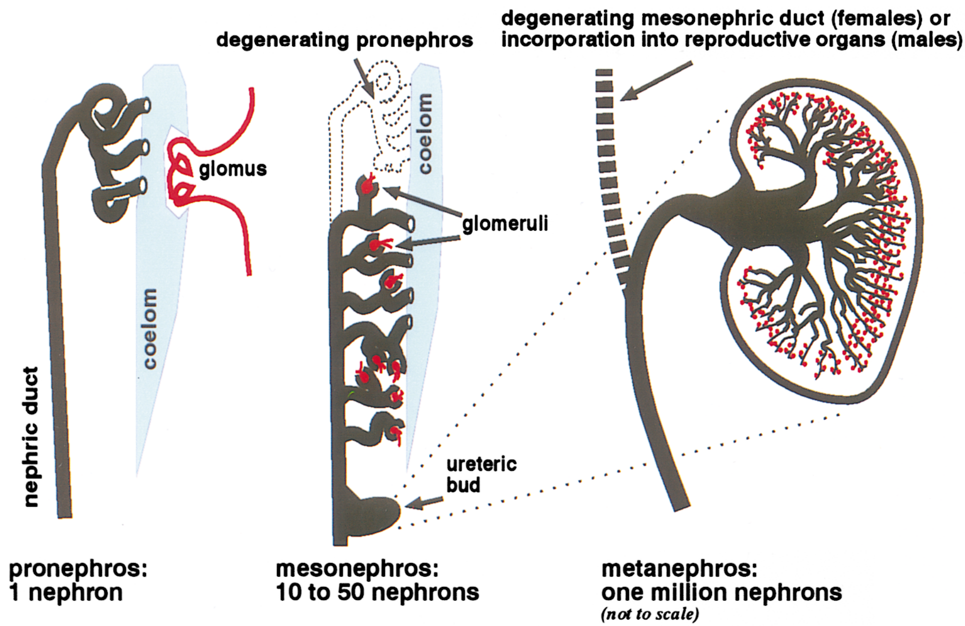


**FIG. 3.** Organization of nephrons into kidneys (based on Goodrich, 1930; and Balinsky, 1970).

aorta, reflecting its dual origins (Tytler, 1988). At the time of fusion the two glomeruli form an hourglass like shape (Tytler, personal communication). Fluids are filtered directly from the glomus into the very small nephrocoel/glomeral space and are collected from there by the ciliated necks of the pronephric tubules (nephrostomes) on either side of the glomus. In nonteleost fish such as *Lepidosiren* and *Protopterus* (lungfish) and in elasmobranchs (sharks and rays) pronephric tubules resemble those found in amphibians. The lungfish pronephros contains a vascularized glomus, while elasmobranch pronephroi do not (Kerr, 1919; Goodrich, 1930).

The vital function of pronephroi has been demonstrated in fish and amphibians both by direct observation of pronephric clearance of injected dye (e.g., Armstrong, 1932) and by pronephric extirpation (e.g., Howland, 1921; Fales, 1935). Bilateral removal of urodele or anuran pronephroi results in edema and death (e.g., Howland, 1921; Fales, 1935; Holtfreter, 1944). The pronephros also plays a major role in hematopoiesis in fish (e.g., Hansen and Kaattari, 1996).

Pronephric tubules are also observed in birds, reptiles, and mammals (Goodrich, 1930), but these organs lack glomera and glomeruli. They may not be functional or may function by collecting wastes excreted into the body cavity by the coelomic epithelium.

## 3. DEVELOPMENT OF THE PRONEPHROS

Although recent kidney reviews tend to concentrate on the morphogenesis of the metanephros, it was not always so. The pronephros was studied by developmental biologists in preference to the metanephros in the first half of this century and it was only when the *in vitro* culture results of Grobstein (1955, 1956, 1957) and chorioallantoic grafts of Wolff and others (see Wolff, 1970, for review) were published did kidney developmental studies focus on the mammalian adult kidney.

The pronephros was first identified by Müller (1829, 1830) and the glomus identified as its vascular component by Bidder (1846; see Adelmann, 1966, for reviews and translations). One of the most detailed pronephric descriptions to date was performed by Herbert Field in 1891. This extensive report contains over 60 beautiful lithographs and three-dimensional reconstructions depicting pronephric development in two anurans, *Bufo* and *Rana,* and one urodele, *Ambystoma.* Another impressive report on pronephric, mesonephric, and Müllerian duct anatomy and development was published by Hall in 1904. A partial list of useful descriptions of pronephric anatomy and development includes Howland (1921), Fales (1935), Fox (1963 and references therein), Jaffee (1954), Hausen and Riebesell (1991), Nieuwkoop and Faber (1994), Vize *et al.* (1995), Carroll and Vize (1996), and the fish references noted above.

Amphibians have been the subject of the greatest amount of pronephric research, and in recent times the anuran *Xenopus laevis* has become the most common amphibian used in experimental research. The urodele *Ambystoma* is also used widely by experimenters investigating migration of the nephric duct. The first morphological indication of *Xenopus* pronephric development is observed at Nieuwkoop and Faber (1994) stage 21 (Fig. 4; Hausen and Riebessel, 1991;

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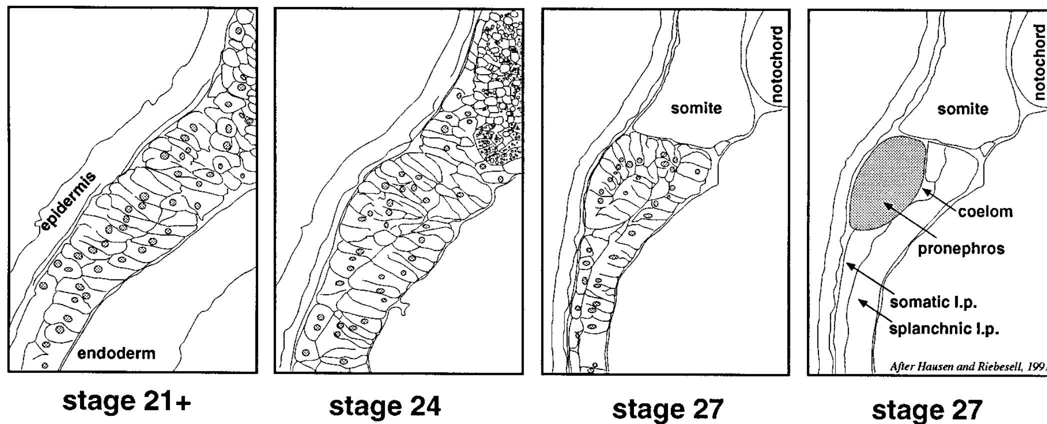


**FIG. 4.** Segregation of the *Xenopus* pronephric anlage from the intermediate mesoderm (redrawn after Hausen and Riebesell, 1991). l.p., lateral plate.

Nieuwkoop and Faber, 1994). This consists of a slight thickening of the somatic portion of the lateral mesoderm below somites 3 through 5. This thickening is caused by cell shape changes as the pronephric precursor cells become more columnar. By stage 24 the pronephric thickening extends back to somite 6 (Nieuwkoop and Faber, 1994) and in transverse sections cells can be seen to be assembling into a compact aggregate that distends the overlying ectoderm. The bump caused by this distension is visible from outside the embryo and is referred to as the pronephric swelling. This mass may be formed by the folding outward of the intermediate mesoderm, forming the outer side of the anlage, and the dorsal region of the somatic lateral plate, forming the inner, ventral side of the anlage. Such a folding of the pronephric mesoderm has been described in a number of organisms (e.g., Goodrich, 1930; Armstrong, 1932) and fits well with the *Xenopus* data of Hausen and Riebesell (1991) reproduced in Fig. 4. At stage 27, the pronephric anlage is a distinct mass that lies distal to the lateral plate, and cells within the anlage are now arranged in a radial fashion. A tiny lumen is first observed in the anterior portion of the anlage at stage 28 and extends to the middle of the anlage by stage 29–30. The entire structure is lumenized by stage 32 (Nieuwkoop and Faber, 1994).

The pronephric duct precursors arise from the posterior–ventral portion of the pronephric anlage, and the duct probably does not form from the fusion of the terminal ends of the pronephric tubules as is reported in many embryology, developmental biology, and anatomy texts. This point was demonstrated by Holtfreter (1944) and Vize *et al.* (1995), both of whom showed that the pronephric duct could form in the complete absence of any pronephric tubules. All of the pronephric tubules and the pronephric duct appear to form from the same outfolding of the intermediate mesoderm (Figs. 2 and 4). Within this precursor structure, cells then segregate into the various components of the pronephros (Nieuwkoop and Faber, 1994).

As discussed above, pronephroi of teleost fish superficially look more similar to metanephric and mesonephric nephrons, but the differences in appearance are due to the separation of the pronephric chamber/nephrocoel from the coelom, the intimate relationship of the neck segment/nephrostome with the pronephric chamber, and the fusion of the early glomeruli into a central glomus (Marshall and Smith, 1930; Tytler, 1988). These differences are minor and the morphogenesis of teleost pronephroi probably follows a sequence very similar to that described above for amphibian pronephroi.

## 4. INDUCTION OF THE KIDNEYS

Most experiments on kidney induction in recent years have investigated the condensation of metanephric mesenchyme in response to the invading ureteric bud (see Saxén, 1987, for review). While this assay is an excellent system in which to analyze the condensation of mesenchyme into epithelia, it is not particularly useful for studying how the kidney primordia are initially specified, as by the time the ureteric bud invades the metanephric mesenchyme many of the key events required for kidney organogenesis have already occurred. As will be discussed below, the metanephrogenic mesenchyme is determined by this stage and lacks only the terminal condensation signals. Pronephric and mesonephric kidney development can be analyzed throughout the period in which the nephric mesoderm is first patterned through to when these simple kidneys differentiate. Such kidneys also have the advantage of less complex and more consistent anatomy and amenability to experimental manipulation, making them ideal systems in which to explore the early stages of kidney specification and determination.

The sequence of inductive interactions required to form each of the kidneys is probably very similar: they are all

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derived from the same embryonic region, the intermediate mesoderm, their nephrons are anatomically very similar, and they perform similar functions. Similar genes are probably involved in the induction of all kidney forms and in later patterning events (see below). There are, however, some differences between meta- and mesonephric and pronephric modes of development. As discussed in Section 3, the pronephric mesoderm ''folds'' away from the intermediate mesoderm and lateral plate without going through a blastemal intermediate, while both meta- and mesonephric nephrons condense from a multipotential mesenchymal cord which has previously segregated away from the intermediate mesoderm. The meta- and mesonephric mesenchymal blastemas are known as the nephrogenic cord. If metanephrogenic cord cells are explanted, their partial specification can be demonstrated by their condensing into tubule epithelia in response to a wide range of heterologous inducing substances (see below). Kidney mesenchyme always forms kidney tubule-like epithelia in such assays. Other explanted mesenchymal cells, for example, salivary gland, do not respond to these inducers in this way and tend to differentiate into epithelia reflecting their own origins (Saxén, 1987). The nephrogenic mesenchyme must therefore be at least partially specified in such explants.

Another difference between pronephroi and other kidneys is the source of the ''terminal'' inductive signal. Pronephroi do not require a signal from the pronephric duct, which forms concomitantly with, or after, the pronephric tubules. The same signaling molecules could be involved in terminal specification of pronephric and mesonephric tubules, but if this is so, these molecules must be produced by other embryonic structures.

As will be discussed below, similar regulatory genes are activated in all kidneys in response to the inductive signals that initiate nephron development. It stands to reason that similar molecules could be performing the equivalent signaling events and that by studying the induction and patterning of any one type of kidney we could learn much about the development of all kidney forms.

Many experiments addressing the nature of meta-, meso-, and pronephric inductive signals have been performed. The metanephric experiments have been extensively reviewed (see Saxén, 1987), so will only be commented upon briefly here. These experiments have demonstrated that a number of different embryonic tissues are capable of triggering the condensation of metanephric mesenchyme into kidney tubules. These tissues include spinal chord (the most effective; Grünwald, 1942, 1943), the ureteric bud epithelia (Grobstein, 1955), and submandibular epithelia, among others (reviewed by Saxén, 1987). More recently, treatment of mesenchymal explants with lithium chloride has been demonstrated to induce tubulogenesis (Davies and Garrod, 1995). These observations indicate that if a specific peptide growth factor mediates the inductive activity of the ureteric bud upon the metanephric mesenchyme, this growth factor may be expressed in a number of embryonic tissues. It is also possible that different intercellular signals which act via the same signal transduction pathway as the natural inducer, or which cross-react with the receptor for the natural inducer, could trigger mesenchymal condensation. Lithium chloride has many effects on cellular metabolism, activating some signal transduction pathways while inhibiting others, and may trigger condensation by mimicking the transduction cascade usually initiated by the endogenous inductive signal.

The ureteric bud, which branches in response to signals from the metanephric mesenchyme, does not respond to any tested heterologous inducing tissues (Saxén, 1987). This would imply that the signal(s) from the metanephric mesenchyme that triggers the outgrowth and branching of the ureteric bud may be very restricted in its spatial distribution. The signal triggering ureteric bud branching is thought to be mediated by the tyrosine kinase receptor c-*ret,* which is expressed in the ureteric bud epithelia (reviewed by Robertson and Mason, 1997). The ligand for c-*ret* is a secreted peptide growth factor, glial-cell-line-derived neurotrophic factor (GDNF), expressed in the metanephric mesenchyme (see below). Presumably, the lack of ureteric bud responses to heterologous inducers was due to none of the tested tissues expressing GDNF.

The tissues responsible for the induction of the mesonephros have been most thoroughly examined in urodeles and birds. Many of these studies investigated the role of the nephric duct (also known as the pronephric, mesonephric, or Wolffian duct and in the older literature by the misleading title of the segmental duct) in this process, based on the important role that the ureteric outgrowth of the nephric duct plays in metanephric development (reviewed by Burns, 1955). Mesonephric tubules are specified by late neurula stages and are determined by tailbud stages (Machemer, 1929). Studies investigating the role of the pronephric duct in this process were probably investigating the final triggering of mesonephric tubule condensation in much the same way as the ureteric bud activates metanephric mesenchyme poised to condense into metanephric nephrons.

The nephric duct migrates posteriorly from the pronephros to fuse with the rectal diverticulum, an anterior outgrowth of the cloaca (O'Connor, 1938, 1939; Holtfreter, 1944; Nieuwkoop and Faber, 1994). Mesonephric tubules form in an anterior to posterior sequence and fuse with the mesonephric duct. The role of the duct in the induction of mesonephric tubules has been investigated by removing the migrating duct prior to it reaching the presumptive mesonephric region (e.g., Burns 1938; Waddington, 1938), by obstructing the posterior migration of the duct (e.g., O'Connor, 1938, 1939; Holtfreter 1944), and by destroying the tip of the migrating duct by cauterization (e.g., Boyden, 1927; Grünwald, 1937). In both amphibians and birds, treatments that disrupted the normal migration or function of the duct either blocked the ability to form mesonephric tubules or resulted in local mesenchymal condensations that failed to form mature tubules. These studies indicate that the mesonephric mesenchyme is largely specified and that a signal from the nephric duct acts much like the ureteric

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bud on the metanephric mesenchyme, i.e., as a terminal inducer.

Etheridge (1968) performed a series of recombinations between intermediate mesoderm explants (presumptive mesonephros) and various other tissue explants to explore what tissues other than the duct were involved in the earlier specification of the mesonephros in urodeles. These experiments were not examining the very first steps in mesonephric specification, as the competent material was taken from late neurula and tailbud stage embryos which was already partially specified (Machemer, 1929), and Etheridge, in fact, observed tubule differentiation in 20 to 40% of his competent samples. The frequency with which tubules formed and the degree to which these tubules developed was enhanced greatly by including additional tissue in the recombinants. Tissues that were found to enhance the differentiation of mesonephric tubules from such competent material included (in order of effectiveness) nephric duct, endoderm, and notochord. Somite was a very weak inducer and neural crest (and crest containing spinal chord) was found to inhibit tubule differentiation (Etheridge, 1968, 1971). Interestingly, the inductive effects of endoderm and notochord were found to be additive. The mesonephric mesenchyme may, therefore, be specified by the combined actions of signals from endoderm and notochord and its final differentiation triggered by the nephric duct. These positive inductive signals may be counterbalanced by negative signals from the neural crest (see above).

There is also a considerable amount of data available on the induction of the pronephros. Fales (1935) demonstrated that the pronephric mesoderm of *Ambystoma* was partially specified by stage 15 (Harrison, 1969). Pronephric mesoderm from stage 15 donor embryos form relatively normal pronephroi when transplanted to the pronephric region of stage 23–25 pronephrectomized hosts. However, when such explants are placed in more posterior positions in these hosts, the transplants develop less well and form smaller pronephroi. This indicates that factors in the vicinity of the pronephros are required to maintain the specification or growth of the presumptive pronephros. Data supporting the existence of such factors in a ''pronephric field'' was also obtained by Holtfreter (1933). In Holtfreter's experiments pieces of gastrula stage ectoderm were inserted under the epidermis of neurula and tailbud stage embryos. The closer the transplants were to the pronephros, the greater the frequency with which they formed pronephric tubules (Holtfreter, 1933; Burns, 1955).

As the pronephros is mesodermal in origin, specification of the pronephros begins with mesoderm induction (reviewed by Kessler and Melton, 1994). Following the induction of mesoderm during early blastula stages and the establishment of the dorsal mesoderm in response to signals generated by cortical rotation (Gerhart *et al.,* 1989), at least two basic states of mesodermal specification are established: ventral mesoderm and dorsal (organizer) mesoderm. Lateral interactions between these two initial classes of mesoderm lead to the establishment of additional types of mesoderm between these two specification classes (reviewed by Slack, 1991). The new specifications include the intermediate mesoderm that will form the heart, kidneys, and gonads. This interaction was demonstrated by Yamada (1937, 1940) and has been reexamined and reviewed by Slack (1991). Yamada (1940) demonstrated that urodele prospective notochord (dorsal mesoderm) will differentiate into notochord if explanted and grown in ectodermal vesicles and that prospective blood rudiments (ventral mesoderm) will differentiate into ventral blood islands when similarly cultured. If prospective notochord is cultured in contact with prospective blood rudiment, the explants will differentiate into notochord, pronephric tubules and blood islands. The ventral explant has thus been respecified to form pronephric tubules by the dorsal mesoderm. Slack and Forman (1980; see Slack, 1991) obtained similar data using *Xenopus* ventral mesoderm explants recombined with *Ambystoma* dorsal mesoderm explants and cultured in a minimal salt solution rather than in ectodermal vesicles.

Yamada (1940) also observed in the course of his experiments that presumptive somite from early neurulae will often form pronephric tubules if explanted and cultured away from the influence of the notochord. Our own studies on pronephric development in embryos ventralized by ultraviolet irradiation also indicate a potential involvement of the somitic mesoderm in pronephric patterning.

As with all inductive assays, caution must be used in interpreting the recombination experiments described above. Not only do many tissues isolated from homologous species and stages exhibit nephric inducing ability, a number of heterologous (and even alcohol treated) tissues can also induce kidney tubules in a variety of assay systems (reviewed by Saxén, 1987). Only when the inducing and patterning genes have been isolated and their activities tested by both ectopic expression and mutation will we truly understand the molecular pathways responsible for regulating kidney development *in vivo.* The availability of molecular markers for different kidney components and effective lineage tracers will also enable experiments investigating the induction of the kidney to be repeated in greater detail and with greater accuracy.

### *Similarities in Induction Patterns of the Different Kidneys*

The existing data on mesonephric and pronephric patterning are very similar with the exception of the involvement of the nephric duct, which appears to play a role in triggering the terminal stages of mesonephric and metanephric differentiation but only forms after the pronephros begins to differentiate, and which is not required for pronephric tubule development (see above). The common implication of the notochord in inducing both pro- and mesonephroi and the enhancement of this induction by endoderm in mesonephroi and by Holtfreter's ''pronephric field'' (endoderm?) in pronephroi implicate these tissues in playing important roles in the patterning of the nephric meso-

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derm. Another intermediate mesoderm derivative, the heart, is also patterned by a combination of dorsal mesodermal and endodermal signals (Sater and Jacobson, 1990; Nascone and Mercola, 1995).

The absence of a role for the duct in triggering pronephric differentiation raises the question of how similar are the induction pathways for the three kidney forms? This remains to be seen, but we would like to predict that they will be very similar, if not identical. A simple molecular hypothesis to explain the reduced importance of the duct in embryonic kidney development is that the signal produced by the duct is identical in nature to that released by one of the inducing tissues and which is present in limiting amounts. Initially the inducing tissue (dorsal mesoderm?) may produce sufficient amounts of the signal to induce the pronephros but lower levels of the signal produced in later stages require additional amounts of the molecule which is also produced by the nephric duct. Mammalian spinal chord may fortuitously produce the same inducing molecule, explaining its ability to trigger metanephric mesenchyme condensation.

Is this relevant to the induction of the metanephric mesenchyme by the ureteric bud? Absolutely. The metanephric mesenchyme must be specified prior to the invasion of the ureteric bud, as the signal for bud growth and branching comes from the mesenchyme, and this is the only known tissue that can trigger the ureteric bud response (Saxén, 1987). The metanephric mesenchyme must be specified by this stage of development to initiate the well-documented interaction between these two tissues. As similar tissues induce pronephroi and mesonephroi, similar genes are activated in all three kidneys in response to these inductive signals (see below) and the nephrons that form go through a very similar pattern of morphogenesis and epithelialization, there is a very good chance that all three kidney types are patterned by similar molecules.

## 5. MOLECULAR REGULATION OF KIDNEY DEVELOPMENT

Traditionally, genes have been identified as playing a role in kidney development on the basis of their expression pattern in murine or avian meta- or mesonephroi, or on the basis of expression pattern and their homology to genes identified as playing important roles in genetic systems. In recent years, the function of genes with these qualifications has been tested by gene ablation using mouse embryonic stem cell technology. Some of the genes implicated as playing a role in kidney development on the basis of expression pattern and sequence homology have been found to be essential for normal metanephric kidney development. Table 1 contains a list of genes demonstrated to play a role in kidney development by gene ablation experiments in the mouse (or other compelling evidence) that are also expressed in the pronephros or the early mesoderm. This table is not a comprehensive listing of genes that are expressed in the kidney or of mutant animals with defects in kidney development. A detailed listing of genes expressed in the kidney, and of mutants with defective metanephric development, can be obtained via the World Wide Web (Kidney Development Database). In all cases tested to date, genes that are required for normal metanephric development are also expressed in pro- and mesonephroi. Where the expression patterns have been studied in detail, the embryonic kidney pattern closely matches that of the adult kidney in as far as their different anatomies (Figs. 1–4) allow. These data indicate that these genes probably play similar roles in the development of all three kidney forms.

The potential role of each of the genes in Table 1 in kidney development, based on expression pattern and the murine mutant phenotype follow.

### *bhh (Banded Hedgehog)*

*Bhh* is a member of the *hedgehog* family of secreted glycoproteins. *Bhh* is expressed in the developing pronephric tubules (Ekker *et al.,* 1995). Although a role for *bhh* has not yet been demonstrated in mammals by targeted gene ablation, the key activities of a related protein (*sonic hedgehog, shh*) in patterning the early embryo implies that this gene may also be important in patterning.

### *BMP-7 (Bone Morphogenetic Protein 7)*

BMP-7 is expressed in the Wolffian duct, mesonephric tubules, and in the comma- and S-shaped bodies of the condensing metanephros (Luo *et al.,* 1995; Lyons *et al.,* 1995; Vukicevic *et al.,* 1996) and is able to induce tubulogenesis in isolated murine metanephric mesenchyme *in vitro* (Vukicevic *et al.,* 1996). BMP-7 mutant mice undergo relatively normal development up until at least Day 12. Few comma- and S-shaped bodies form in the developing metanephros, and condensing mesenchyme is often absent from the tips of the branched ureteric bud (Dudley *et al.,* 1995; Luo *et al.,* 1995). BMP-7 is expressed widely in the entire marginal zone of early *Xenopus* embryos, including in the mesoderm fated to form the pronephros. High-level expression is not observed at later stages during pronephric differentiation (Hawley *et al.,* 1995).

### *DCoH (Dimerization Cofactor of HNF1)*

DCoH is a cofactor that stabilizes LFB1 and LFB3 (see below) and enhances their transcriptional activity, and also acts as a cytoplasmic enzyme, 4a-carbinolamine dehydratase, in the liver. In the pronephros, expression is observed in the pronephric tubules only (Strandmann and Ryffel, 1995). 4a-Carbinolamine dehydratase is expressed in the adult rat kidney, but has not yet been characterized in detail (Davis *et al.,* 1992).

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**TABLE 1**
Pronephric Expression of Metanephric Genes

| | Expression | | | | Mutant phenotype | References |
|---|---|---|---|---|---|---|
| | Pro- | Duct | Meso- | Meta- | | |
| *bhh* | + | − | ? | ? | ? | Ekker *et al.*, 1995 |
| BMP-7 | +* | + | + | + | No metanephros | Hawley *et al.*, 1995; Dudley *et al.*, 1995; Luo *et al.*, 1995 |
| DCoH | + | − | ? | + | ? | Strandmann and Ryffel, 1995 |
| GDNF | ? | − | ? | + | No metanephros | Robertson and Mason, 1997 |
| HGF | + | ? | ? | + | Early lethal | Nakamura *et al.*, 1995; Uahara *et al.*, 1995; Schmidt *et al.*, 1995 |
| *ld* (formin) | + | + | + | + | No metanephros | Trumpp *et al.*, 1992; Mass *et al.*, 1994 |
| *lim*-1 | + | + | + | + | No metanephros | Taira *et al.*, 1994a; Shawlot and Behringer, 1995 |
| LFB1/HNF1a | + | − | + | + | Renal dysfunction | Weber *et al.* 1996; Pontoglio *et al.*, 1996 |
| LFB3/HNF1b | + | + | + | + | ? | Demartis *et al.* 1994 |
| c-*met* | + | + | + | + | ? | Our observations; Woolf *et al.*, 1995 |
| N-myc | + | − | ? | + | Small metanephros | XMMR, 1996; Moens *et al.*, 1993 |
| *Pax*-2 | n.a. | + | + | + | No metanephros | Our observations; Torres *et al.*, 1995 |
| *Pax*-b | + | + | + | n.a. | n.a. | Krauss *et al.*, 1991; our observations |
| PDGF-B | ? | ? | ? | ? | Glomeruli lack mesangium | Leveen *et al.*, 1994 |
| PDGF-BR | ? | ? | ? | ? | Glomeruli lack mesangium | Soriano, 1994 |
| c-*ret* | Low | + | + | + | No metanephros | Our observations; Schuchardt *et al.*, 1994 |
| *wnt*-4 | + | − | + | + | No metanephros | Our observations; Stark *et al.*, 1994 |
| *WT1* | + | − | + | + | No metanephros | Carroll and Vize, 1996; Kreidberg *et al.*, 1993 |

*Note.* Transcription of kidney genes in pronephric (pro-), nephric duct (duct), mesonephric (meso-), and metanephric (meta-) tubules is indicated. Nephric duct includes pronephric duct, Wolffian duct, or ureteric bud. n.a., not applicable. The *Pax*-b gene is probably an ancestral gene present only in lower vertebrates which possess only pro- and mesonephroi. Likewise, *Pax*-2 is probably a decendant of *Pax*-b, and is therefore only present in higher vertebrates which do not possess a well developed pronephros (Carroll and Vize, in preparation). Mutant phenotype, the phenotypic consequence of targeted mutagenesis in developing mice. ?, unknown, +*, expression of BMP-7 has been observed in entire marginal zone, not in the pronephric mesoderm itself (Hawley *et al.*, 1995). Caution should be used in ascribing gene expression in a lateral ridge below the somites to the migrating pronephric duct, as early broad expression throughout the intermediate mesoderm has been observed in some genes that are never expressed in the migrating pronephric duct (Carroll and Vize, in preparation). Likewise, the posterior cardinal vein in *Xenopus* lies adjacent to the posterior pronephric duct and can easily be confused with duct staining. For gene name abbreviations, see text.

### *GDNF (Glial Cell-Line Derived Neurotrophic Factor)*

GDNF is a peptide growth factor originally isolated from media conditioned by glial cells and has been widely studied due to its potent neurotrophic and cell survival activities. The metanephric mesenchyme expresses GDNF which then triggers the formation of the ureteric bud via the c-*ret* tyrosine kinase receptor (reviewed by Robertson and Mason, 1997). *Xenopus* ectodermal explants containing ectopic c-*ret* form mesoderm when treated with soluble GDNF (Durbec *et al.*, 1996).

### *HGF (Hepatocyte Growth Factor)*

HGF is a mitogenic growth factor required for liver development and for normal blood vessels. Mice mutant for HGF die very early, so later defects in kidney development or vascularization cannot be determined (Uahara *et al.*, 1995; Schmidt *et al.*, 1995). Antibodies against HGF block metanephric condensation in explants containing both mesenchyme and bud, and HGF can trigger epithelialization of metanephric mesenchyme isolated from the influence of the ureteric bud (Woolf *et al.*, 1995). HGF is expressed widely in the developing frog embryo, including in somites and pronephric anlage (Nakamura *et al.*, 1995). HGF is the ligand for the c-*met* tyrosine kinase.

### *ld (Formin) (limb deformity)*

The formins are a group of nuclear proteins required for normal limb and kidney development. Chick *ld* is expressed in pronephric and mesonephric tubules and in the nephric duct (Trumpp *et al.*, 1992), and murine *ld* is expressed in both the metanephrogenic mesenchyme and ureteric bud (Maas *et al.*, 1994). Kidney development in mouse *ld* mutants fails due to the lack of ureteric bud outgrowth. It is not yet clear if this failure is due to the bud failing to receive signals from the mutant mesenchyme (which also normally

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expresses *ld*) or to a defect in the nephric duct itself (Maas *et al.,* 1994).

### LFB1/HNF1α (Liver Factor B1/Hepatocyte Nuclear Factor-1α)

LFB1 is a homeodomain containing transcription factor and is expressed in multiple tissues including pro-, meso-, and metanephroi (Weber *et al.,* 1996). Expression of LFB1 in metanephroi appears later than that of LFB3 and is restricted to proximal and distal tubules. Mutant mice (Pontoglio *et al.,* 1996) develop to term but have enlarged livers and kidney defects reminiscent of renal Fanconi syndrome (glucose and water loss due to dysfunction of proximal tubules).

### LFB3/HNF1β (Liver Factor B3/Hepatocyte Nuclear Factor-1β)

LFB3 is also a homeodomain containing transcription factor and is expressed in multiple tissues including pro-, meso-, and metanephroi (Demartis *et al.,* 1994). In metanephroi the expression of LFB3 is observed in kidney mesenchyme as it first begins to condense, and expression is observed in both tubules and collecting duct (Lazzaro *et al.,* 1992). The metanephric pattern of expression of LFB1 and LFB3 is consistent with that observed in pronephroi, where LFB1 is only observed in the pronephric tubules while LFB3 is found in both pronephric tubules and pronephric duct (Demartis *et al.,* 1994; Weber *et al.,* 1996).

### lim-1

A homeobox and LIM domain containing protein required for anterior patterning in the mouse and also required for kidney organogenesis. The basis of the kidney defect is unknown. Ectopic expression of mutant Xlim-1 in frog (Taira *et al.,* 1994b) induces an ectopic dorsal axis (additional head), while ablation in the mouse results in a failure to pattern the head or to form a meta- or mesonephros (Shawlot and Behringer, 1995). In frog embryos, *Xlim*-1 is activated in the prepronephric region immediately following gastrulation and is one of the earliest markers of nephrogenic potential (Taira *et al.,* 1994a).

### c-met (the Tyrosine Kinase Receptor Activated by HGF)

C-*met* is related to c-*ron,* the receptor activated by the HGF-like protein. The role of c-*met* in the kidney is unknown; however, as HGF has been implicated in kidney development by multiple lines of evidence (Woolf *et al.,* 1995), it seems likely that c-*met* must play a key role. C-*met* is expressed widely in the developing embryo, including in the developing pronephric tubules, pronephric duct, and mesonephros (XMMR).

### N-myc (a Member of the myc Family of Proto-oncogenes)

Targeted mutation of N-*myc* in mice results in smaller than normal kidneys (Moens *et al.,* 1993). N-*myc* can control proliferation rates in cultured cells, and may be required for normal cell division rates in the kidney. In the pronephros expression is observed in the pronephric anlage (*XMMR*), but has not been characterized in detail.

### Pax-2 (Paired Box-2)

*Pax*-2 is expressed in the condensing metanephric tubules and in the ureteric bud epithelium. Murine *Pax*-2 mutants lack both ureteric bud (and the entire Wolffian duct), and the meso- and metanephric mesenchyme fails to epithelialize (Torres *et al.,* 1995). *Pax*-2 is also expressed in the mesonephric tubules and nephric duct.

### Pax [zf-b]/Pax-B (Paired Box-B)

This gene is probably related to the ancestor of the Pax-2 and Pax-8 genes, and is only found in fish and amphibians (in which no Pax-2 or Pax-8 genes have been identified). *Pax*-B is expressed in pro- and mesonephric tubules, nephric duct, and rectal diverticulum (Krauss *et al.,* 1991). This gene is not expressed in metanephroi as lower vertebrates do not have this form of kidney.

### PDGF-B (Platelet-Derived Growth Factor-B)

PDGF-B is a peptide growth factor expressed in many cell types during normal development, including endothelial cells (Raines *et al.,* 1990). In murine PDGF-B mutants, abnormal glomeruli lacking mesangial cells form (Levéen *et al.,* 1994). Mesangial cells are endothelial cells that support the blood vessel endothelia of the glomeruli (Hyink and Abrahamson, 1995). The blood vessels in such glomeruli collapse and the Bowman's space fills with blood cells. The expression of PDGF-B in pronephroi has not been described and is included in this table due to its potential role in glomeral development.

### PDGFB-R (Platelet-Derived Growth Factor-B Receptor)

PDGFB-R is the tyrosine kinase receptor for PDGF-B homodimers and PDGF-A/PDGF-B heterodimers. This receptor is normally expressed in multiple cell types, including smooth muscle and endothelial cells (Ross *et al.,* 1986). Murine PDGFB-R mutants have abnormal glomeruli lacking mesangial cells (Soriano, 1994) similar to those observed in PDGF-B-deficient mice (see above). Together these results argue for a key role of PDGF-B signaling in the development of the glomerular mesangium.

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### *c-ret* (a Transmembrane Tyrosine Kinase with an Extracellular Domain with Homology to Cadherins)

This protein functions as a cellular receptor for GDNF. It is required in the ureteric bud (Schuchardt *et al.,* 1994) to initiate bud outgrowth in response to mesenchymal (GDNF) signals. C-*ret* is expressed at highest levels in the branching ureteric bud in metanephroi and tip of the growing pronephric duct in pronephroi (XMMR). Ectopic expression of a constitutively active form of human c-*ret* can trigger the formation of pronephric epithelia in naive *Xenopus* ectoderm (Durbec *et al.,* 1996).

### *wnt-4* (wingless/int Related 4)

*Wnt*-4 encodes a secreted glycoprotein related to the *Drosophila wingless* gene and mammalian *int*-1 proto-oncogene. The murine *wnt*-4 mutant phenotype includes the failure of late stages of metanephric mesenchyme condensation (Stark *et al.,* 1994). In such mutant kidneys most mesenchymal genes (e.g., *Pax*-2 and *WT*-1) are activated normally. In metanephroi, *wnt*-4 expression is normally highest in the mesenchyme adjacent to the branching tips of the ureteric bud. In pronephroi, expression is strongest at the dorsal tips of the growing pronephric tubules (XMMR).

### *WT1* (Wilms' Tumor Suppressor Gene-1)

*WT1* is found to be mutated in 10% of Wilms' tumors (Hastie, 1994), pediatric tumors of the urogenital system (Wilms, 1899). A zinc-finger-type transcription factor closely related in sequence to EGR/zf268 (a mitogen-activated transcription factor). Expression of *WT1* in cultured cells can prevent apoptosis (Maheswaran *et al.,* 1995). Mutation of *WT1* in mice results in apoptosis of the metanephrogenic mesenchyme (Kreidberg *et al.,* 1993). Surprisingly, at least some mesonephric tubules appear to form normally in these mutant mice (Sainio *et al.,* 1997). It has been proposed that the surviving mesonephric tubules in such mice may develop in a manner more analogous to pronephric tubules or possibly even represent pronephric tubules (Sainio *et al.,* 1997). In metanephroi low-level expression of *WT1* is initially observed throughout the nephric mesenchyme. Later high-level expression is observed in the podocytes of the glomeruli (Mundlos *et al.,* 1993). In the pronephros, expression is observed in the glomus, the equivalent of the metanephric glomerulus, but is not observed in the pronephric tubule anlage (Fig. 1; Carroll and Vize, 1996). It is quite possible that *WT1* plays a common role in glomerular development in all kidney forms, and that the more complex kidneys utilize this gene in a second manner in the nephrogenic mesenchyme. In mice *WT1* function appears to only be essential for kidney components derived from such mesenchyme. It will be fascinating to determine whether *WT1* function is essential for pronephric development.

These similar expression patterns in multiple kidney forms clearly indicate that a similar genetic cascade operates in all kidneys. It would seem to be extremely unlikely that different signals activate the same downstream genes in each kidney. We conclude that genes demonstrated to play important roles in any one kidney system will probably play similar roles in other forms, although, as the *WT1* data indicate, there may well be interesting exceptions to this rule.

## 7. WHY HAVE DIFFERENT KIDNEY FORMS?

The data presented above indicate that all three vertebrate kidney forms perform the same function, have similar nephrons (with the exception of internal versus external glomeruli/glomera), are all derived from the intermediate mesoderm, and all use similar patterning genes. So why reiterate organogenesis two or three times during the life of the organism? There are four possible reasons: function, time, evolutionary dependance, and evolutionary baggage (Smith, 1943; Smith, 1953).

The first reason, function, would argue that the differently organized kidneys do in fact function in somewhat different manners. For instance, the simple kidneys, with their open connections to the coelom and cilia-driven fluid movement, may function better with the very low blood pressures found in early embryos (Girard, 1973). Although the metanephros is an incredibly efficient organ, its function depends on a high arterial blood pressure. Given the limitations of the embryonic heart and vascular system, simple ciliated kidneys may be the most functional kidney form at this stage of development. This possibility is supported by the observation that the early mesonephric tubules in lower vertebrates contain both nephrostomes and glomeruli, while tubules that form once the circulatory system is well developed contain only glomeruli (Goodrich, 1930).

The second possibility, time, also seems possible. Early embryos need some form of functional kidney or they die from a failure to control water balance (Howland, 1921). It would seem much easier to form a pronephros, which is in essence one large super-nephron, than it would be to form a more complex meso- or metanephros in a short period of time. The *Xenopus* pronephros takes only 31 hr to become functional after the first morphological signs of its formation are apparent (Nieuwkoop and Faber, 1994). The *Xenopus* mesonephros, however, takes 5 days for the first set of tubules to become functional from when their anlage is first observed ($7\frac{1}{2}$ days total), and it takes an additional 39 days for the mesonephros to reach its full size (Nieuwkoop and Faber, 1994). As pronephric function is required after 3 days of embryonic development, the advantages of a simple temporary kidney are obvious: provide time for a complex kidney to form.

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The third possibility is evolutionary dependance. The acquisition of integrated glomeruli and the more complex nephron organization observed in meso- and metanephroi are variations on earlier themes that evolved in the vertebrates and are also used by elasmobranchs (Smith, 1943, 1953). So why do organisms that have evolved more complex and efficient kidneys still utilize more primitive embryonic kidneys? Why hasn't natural selection disposed of the ''redundant'' simple kidneys? The functionality and timing arguments outlined above are two strong possibilities. Another possible reason is the mechanism by which the more complex kidneys evolved. Both mesonephroi and metanephroi utilize the pronephric duct, or derivatives of the pronephric duct, to dispose of urine. If the pronephros were to disappear from these organisms they would have to develop a new duct system. Likewise, as discussed in Section 4, the pronephric duct has been strongly implicated to be the terminal inducer of the mesonephric mesenchyme, and an outgrowth of the pronephric duct, the ureteric bud, is absolutely required for induction of the metanephric mesenchyme. Once again, if the simple kidneys were disposed of by evolution, the inducers of the more complex kidneys would have to be reinvented or redeployed.

A forth possible reason, favored by much of the literature, is that the pronephros is simply a nonfunctional remnant of evolutionary history that has not been disposed of yet (see Smith, 1953; Torrey, 1971). In our view, some combination of the first three arguments outlined above is more likely to explain the preservation of the unusual mode of development utilized by the nephric system.

## 8. SUMMARY AND PROSPECTS

The similar gene expression patterns in all three kidney forms argue that similar pathways of development, function, and induction have been conserved (Table 1). Many authors have previously discussed both the anatomical and functional similarities between different kidney forms (see Torrey, 1971) and the existence of intermediate forms that defy classification as one of the three kidneys discussed here (see Goodrich, 1930). The different kidney forms are part of a continuum, with different parts being dominant at different stages of development. In this review we have presented molecular data to support this contention. When molecular differences are in fact observed between different kidneys, this data will be useful in identifying genes involved in kidney form-specific features. One such potential difference has already been noted: *WT1* is essential for metanephric tubule development, but some mesonephric tubules form in its absence (Kreidberg *et al.,* 1993; Sainio *et al.,* 1997). Thus, even though *WT1* is expressed in pro- and mesonephroi (Carroll and Vize, 1996) it may function differently in kidneys derived from the direct folding of the intermediate mesoderm and in those derived from a multipotential mesenchymal blastema (Sainio *et al.,* 1997).

We argue that each kidney form is an excellent system in which to investigate general principles of the development of the nephric system. The advantages of each form can be used to explore genetic cascades and inductive interactions which will probably apply to all other forms. Targeted mutagenesis can be used in the mouse to explore gene function in the metanephroi, organ culture combined with retroviral transfection systems can be used to explore gene function and inductive interactions in chick mesonephroi, and genetics (zebrafish), experimental embryology, and microinjection techniques (*Xenopus*) can be used to dissect the development of pronephroi. In the next decade the combined application of these techniques may begin to unravel the secrets of organogenesis. As all forms of kidney, including pro- and mesonephroi, are susceptible to various forms of cancer (Hard, 1984; Carlson *et al.,* 1995; Wallingford, Seufert, Virta, and Vize, submitted for publication) such experiments will also be of great value to the study of nephric carcinomas and blastomas. The availability of additional experimental systems, namely, the simple kidneys of the lower vertebrates, can only add to the chances of success in these endeavors.

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