Towards a molecular anatomy of the *Xenopus* pronephric kidney

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Introduction

The building of organs during embryonic life constitutes one of the most fascinating, but also least understood developmental processes. The assembly of organs from a small pool of embryonic cells to a complex three-dimension structure with characteristic shape and size, defined structural composition and specialized physiological properties, is the result of coordinated gene action that directs the developmental fate of cells participating in the process. The acquisition of different cell fates initiates an intricate interplay of cell proliferation, migration, growth, differentiation, and death, elaborating and bringing together cellular ensembles in a precise temporal and spatial manner. Intrinsic, cell-autonomous factors, as well as non-autonomous, short-range and long-range signals underlie specification, pattern formation, and inductive interactions that guide cells along distinct developmental pathways. How intrinsic and extrinsic factors are integrated to generate cell diversity, coordinate morphogenetic cell movements, and regulate assembly of the different tissue types comprising an organ, defines one of the central questions in developmental biology.

The mammalian kidney has long served as an important model for studying numerous problems associated with organogenesis (Saxén, 1987; Lechner and Dressler, 1997; Davies and Bard, 1998; Müller and Brändli, 1999). Although the adult kidney is a fairly complex organ, its morphogenesis seems to be relatively simple involving a set of developmental mechanisms common to many other organ systems. These include specification of stem cells, mesenchyme-to-epithelial transitions, branching morphogenesis, tubulogenesis, patterning along the length of an epithelial tubule, and vascularization. The development of the mammalian kidney has been studied primarily in rodents since an organ culture system permitting the *in vitro* analysis of kidney induction

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at the molecular and cellular level is available (Grobstein, 1953; Saxén, 1987). More recently, transgenic mouse mutants with severe renal malformations have complemented these studies (Lechner and Dressler, 1997; Vainio and Müller, 1997). Most impressively, the combination of mouse genetics with a powerful organ culture system has led to the identification and characterization of the key components (GDNF, c-Ret, GFRA1) of a signaling pathway that promotes branching morphogenesis during kidney development (Sariola and Sainio, 1997; Davies and Bard, 1998). Several important questions still remain unresolved. How are the different cell lineages of the kidney specified? What are the inductive signals that promote aggregation and epithelial conversion of nephrogenic mesenchyme? How is segmental gene expression along renal tubules achieved?

It should be stressed that, to date, most attention has been focused on understanding the late steps of kidney organogenesis leading to the formation of the metanephric kidney. The first epithelial tubule to differentiate from the mesoderm is however the pronephric duct. It is formed in one of the first mesenchymal-to-epithelial conversions and is required for all further steps of kidney development (Saxén, 1987; Herzlinger, 1995). Despite its essential role, the regulation of pronephric duct formation in early embryogenesis remains poorly characterized. Similarly unresolved is the question what specifies the early kidney primordia of the mesoderm. The answers to these questions are not readily amenable in mammalian systems. Lower vertebrates, primarily the frog *Xenopus laevis*, have therefore replaced rodents as the preferred subject for research on earlier stages of kidney development (Vize et al., 1997; Carroll et al., 1999a). The *Xenopus* model system is ideally suited to study the development of the pronephric kidney, the first excretory system established during vertebrate embryogenesis. Organ development occurs rapidly. A fully functional pronephric kidney is established within little more than two days post fertilization (Nieuwkoop and Faber, 1994). *Xenopus* embryos can be manipulated with relative ease permitting gain- and loss-of-function studies. Furthermore, pronephric cell lineages have been isolated and an explant culture system permits *in vitro* induction of nephron formation. In this review, the structural organization, the development *in vivo* and *in vitro*, and the cellular diversity associated with the *Xenopus* pronephric kidney will be described. Recent progress in identifying novel marker genes and systematic analysis of the temporal and spatial expression patterns has generated the contours of an emerging molecular anatomy of the pronephric kidney. Given the many similarities between pronephric and metanephric kidney development, future investigations should permit the dissection of pronephric gene function and lead to the identification of key genetic cascades needed to establish the renal function.

**Structural organization of the pronephric kidney**

Kidney development is characterized by the successive formation of three sets of spatially and temporally different embryonic excretory organs: the pronephros, the mesonephros, and the metanephros (Saxén, 1987; Vize et al., 1997). Pronephros and mesonephros are only transiently present during early embryonic life of mammals, and the permanent kidney develops from the metanephros. In fish and amphibia, the pronephros is the fully functional embryonic kidney and indispensable for larval life. The pronephros will undergo regression and apoptosis and its function will be replaced by the mesonephros, which will form the mature kidney of lower vertebrates.

All three kidneys share a similar basic structural organization and differ mostly in the number and spatial assembly of the nephrons, the functional units of vertebrate kidneys. The generic vertebrate nephron consists of three major components: the renal corpuscle, the renal tubule, and the renal duct. The renal corpuscle is responsible for blood filtration and is a combination of two structures: the vascular loops of the glomerulus and the renal capsule. The visceral (or podocyte) layer of the renal capsule invests the capillaries of the glomerulus. The visceral layer is continuous with the parietal layer, and together they constitute the renal capsule proper. The space between the visceral and parietal layers is known as the nephrocoel (or urinary space), which is continuous with the lumen of the renal tubule. The renal tubule extends from the renal capsule to its junction with the renal duct. It is lined by a single layer of epithelial cells that function in selective reabsorption of water, inorganic ions, and other molecules from the glomerular filtrate. The renal duct communicates with the exterior and serves as the exit channel for the remaining waste products.

In contrast to the metanephros, the pronephros is a relatively simple organ. The pronephros of fish and amphibia typically contain 1-3 nephrons (using the number of renal tubules as the defining criteria), whereas metanephros can have up to 1 million nephrons (Saxén, 1987). The pronephros is derived, like the more advanced kidneys, from the intermediate mesoderm, which lies lateral to the somites. The basic design of the *Xenopus* pronephric kidney is shown in Figure 1. The pronephros is composed of three principle parts: the pronephric corpuscle, the pronephric tubules, and the pronephric duct. Recently, the term “glomerus (plural glomera)” has been frequently used to refer to the filtration unit of the pronephros. Strictly speaking, only the vascular structures of a renal corpuscle that extend over multiple body segments or contain multiple fused glomeruli may be referred to as glomera (Goodrich, 1930; Balinsky, 1970). More accurately, the pronephric filtration unit is therefore termed the pronephric corpuscle (Felix and Bühler, 1906; Balinsky, 1970). The *Xenopus* pronephros contains a single corpuscle, which consists of the pronephric capsule and a vascular component, the glomus (Fig. 1A). The visceral layer of the pronephric capsule contacting the glomus develops as a pocket of splanchnic intermediate mesoderm that protrudes into the nephrocoel, the filtration chamber of the pronephros. The nephrocoel and the coelom are initially contiguous but later separate into distinct cavities (Vize et al., 1997). The nephrocoel is lined by both visceral and parietal epithelium. The capillary network forming the glomus is derived from the dorsal aorta (Nieuwkoop and Faber, 1994). It is likely that podocytes and endothelial cells of the *Xenopus* pronephros form a basement membrane, similar to the trilaminar glomerular basement membrane found in zebrafish pronephroi (Majumdar and Drummond, 1999). Pronephric tubules are composed of at least three morphologically distinct segments: ciliated nephrostomes, connecting tubules, and a common tubule (Fig. 1B). Each connecting tubule is linked to the common tubule, which connects to the pronephric duct. Fusion of the pronephric duct with the rectal diverticulum, an outgrowth of the cloaca, links the pronephric kidney to the exterior. The convoluted pronephric tubules are permeated with venous blood vessels that arise in close association with the posterior cardinal vein to form the pronephric sinus.
Development of the pronephric kidney

The intermediate mesoderm located lateral to the somites gives rise to all three forms of the vertebrate kidney (Saxén, 1987). It generates nephrogenic mesenchyme and the nephric (or Wolffian) duct epithelium, the principle player directing nephrogenesis. The successive appearance of the pronephric, mesonephric, and metanephric nephrons is the result of an antero-posterior wave of cellular differentiation in the nephrogenic mesenchyme and depends on inductive interactions with the nephric duct epithelium and its derivative, the ureteric bud. In response to duct-derived signals, nephrogenic mesenchyme undergoes simultaneously mesenchyme-to-epithelial conversion to form nephric tubules and differentiation to generate stromal cells. When nephric duct elongation is prevented, mesonephric and metanephric nephrons do not form from intermediate mesoderm. Furthermore, isolated intermediate mesoderm undergoes programmed cell death in absence of an inducer (Herzlinger, 1995). Differentiation and morphogenesis of mesonephrogenic and metanephrogenic mesenchyme is therefore determined by the nephric duct epithelium, which rescues cells from entering the apoptotic pathway. The signal that passes from the nephric epithelium to the nephrogenic mesenchyme is arguably the key event in kidney development, yet its molecular nature has still to be determined.

The nephric duct constitutes the central component of the excretory system throughout renal development, but the molecular and cellular interactions regulating its formation are poorly understood. The nephric duct, initially called the pronephric duct, is formed along with the other components of the pronephric kidney from the anterior intermediate mesoderm. The morphogenetic events leading to the mature pronephric kidney and its derivatives are currently best understood in amphibian embryos (Fox, 1963; Nieuwkoop and Faber, 1994; Vize et al., 1997; Carroll et al., 1999a). The anterior intermediate mesoderm is initially continuous with the double-layered sheet of lateral plate mesoderm. With continuing development, a morphologically detectable, separate entity becomes apparent, which will go on to form the functional units of the pronephric kidney. The two layers of the intermediate mesoderm will give rise to distinct components of the pronephric kidney. The outer layer facing the epidermis represents the somatic layer and will generate pronephric tubules and duct. The inner (splanchnic) layer adjacent to the endodermal yolk mass, will form the pronephric capsule. In *Xenopus*, cells of the somatic layer below somites 3 to 5 will start condensing at around stage 21 (Nieuwkoop and Faber, 1994; Vize et al., 1997). They will give rise to the pronephric tubule anlage, which will later generate epithelia of the nephrostomal funnels, the connecting tubules, and the common tubule. The process of pronephric tubule formation requires changes in cell shape and extensive cell rearrangements similar to those seen during mesenchyme-to-epithelial conversion of metanephrogenic mesenchyme. Remarkably, similarities between these two morphogenetic processes are not only seen at the cellular, but also at the molecular level as illustrated by expression of Wnt-4, a regulator of tubulogenesis (see below).

Unlike the other pronephric tubule epithelia, the pronephric duct epithelium is believed to have a separate developmental origin. It is thought to arise from the pronephric duct anlage, which forms by condensation of a segment of somatic intermediate mesoderm located in *Xenopus* below somites 5-7 and thus positioned caudal to the pronephric tubule anlage (Nieuwkoop and Faber, 1994; Vize et al., 1997).
et al., 1997). Separate primordia for tubules and duct are postulated based on findings from experiments where presumptive pronephric anlagen were dissected (Holtfreter, 1944; Vize et al., 1995). At present, it is not known with certainty whether pronephric tubules are formed independent of signals derived from the pronephric duct anlagen. Although specification of pronephric tubules appears to occur prior to the pronephric duct (Brennan et al., 1998), this does not exclude the possibility of the duct anlage promoting some aspects of tubular differentiation. From stage 26 onward, the Xenopus pronephric duct extends in posterior direction along a pathway immediately ventral to the developing somites. The extension process continues until by stage 36/37, when fusion with the rectal diverticulum occurs (Nieuwkoop and Faber, 1994; Heller and Brändli, 1997). Elongation of the pronephric duct involves active cell migration (Lynch and Fraser, 1990; Drawbridge and Steinberg, 1996). The molecular nature of the cues guiding directed migration of pronephric duct cells is currently not known. Pronephric duct migration is sensitive to the removal of polysialic acid moieties or digestion with PI-PLC suggesting roles for glycoproteins and GPI-linked proteins (Zackson and Steinberg, 1989; Bellairs et al., 1995). Similarly, the signals controlling the assembly of pronephric duct cells into an epithelial tubule are poorly understood. Recent findings demonstrate however that the surface ectoderm overlying the pronephric duct primordium is required for nephric duct formation in the chicken embryo and that BMP-4 signaling plays a central role in this process (Obara-Ishihara et al., 1999).

The development of the pronephric corpuscle (capsule and glomus) in Xenopus has not been thoroughly investigated. The pronephric capsule anlage is located in the splanchnic mesoderm. Transplantation experiments performed with urodeles suggest that the pronephric anlage acts as the inducer of the pronephric capsule (Fales, 1935). Recent findings in zebrafish rule out the possibility that endothelial cell-derived signals direct the formation and differentiation of the pronephric capsule (Majumdar and Drummond, 1999). A role for the underlying endoderm can however not be excluded at present. Onset of pronephric capsule morphogenesis occurs considerably later than pronephric duct and tubules form. The glomus appears at stage 29/30 as a small and compact bud of capillaries that has sprouted from the dorsal aorta (Nieuwkoop and Faber, 1994), possibly through an angiogenic mechanism. Glomus and splanchnic intermediate mesoderm form a fold that extends into the nephrocoel. Both glomus and the filtration chamber gradually increase in size until blood supply starts at stage 35/36 (Nieuwkoop and Faber, 1994). The time point when differentiation of the pronephric capsule epithelium into podocytes (visceral epithelium) and parietal epithelium occurs is not known.

**TABLE 1**

**USEFUL MARKERS FOR DIFFERENT CELL LINEAGES IN THE DEVELOPING XENOPUS PRONEPHRIC KIDNEY**

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Marker gene</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early pronephric anlage</td>
<td>Pax-8</td>
<td>Heller and Brändli, 1999</td>
</tr>
<tr>
<td></td>
<td>HNF-1β</td>
<td>Demartis et al., 1994</td>
</tr>
<tr>
<td>Tubule anlage and tubule epithelia only</td>
<td>Wnt-4</td>
<td>Mc Grew et al., 1992; D.M.E. Saulnier and A.W. Brändli, manuscript in preparation</td>
</tr>
<tr>
<td>Duct anlage and duct epithelia only</td>
<td>Pou-2</td>
<td>Witta et al., 1995; D.M.E. Saulnier and A.W. Brändli, manuscript in preparation</td>
</tr>
<tr>
<td>Tubule and duct epithelia, rectal diverticulum</td>
<td>Pax-2</td>
<td>Heller and Brändli, 1997</td>
</tr>
<tr>
<td>Rectal diverticulum only</td>
<td>WIF-1</td>
<td>Hsieh et al., 1999</td>
</tr>
<tr>
<td>Pronephric capsule anlage and visceral (podocyte) epithelium</td>
<td>WT-1</td>
<td>Carroll and Vize, 1996; Semba et al., 1996</td>
</tr>
<tr>
<td>Hemangioblasts, hematopoietic progenitors</td>
<td>SCL</td>
<td>Mead et al., 1998</td>
</tr>
<tr>
<td>Angioblasts, endothelia</td>
<td>Msr</td>
<td>Devic et al., 1996</td>
</tr>
<tr>
<td></td>
<td>Flk-1</td>
<td>Cleaver et al., 1997</td>
</tr>
<tr>
<td>Hematopoietic progenitors</td>
<td>GATA-2</td>
<td>Kelley et al., 1994; Bertewistle et al., 1996</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>αT4 larval globin</td>
<td>Walmsley et al., 1994</td>
</tr>
<tr>
<td>Trunk neural crest cells</td>
<td>PDGFRα</td>
<td>Jones et al., 1993; M. Mercola, personal communication</td>
</tr>
</tbody>
</table>
Recently, it was demonstrated that the induction of pronephric tubules could also be reproduced in an organ culture system prepared from presumptive ectoderm of Xenopus embryos (Moriya et al., 1993; Uochi and Asashima, 1996). The explants (‘animal caps’) dissected from the animal pole of blastula stage embryos have been successfully used to identify factors that mediate mesoderm induction and patterning (Dawid, 1994; Kessler and Melton, 1994; Slack, 1994). In these cultures, the growth factor activin can induce in a dose-dependent manner differentiation of animal cap cells into all mesoderm derivatives, but pronephric tissue (Asashima, 1994; Dawid, 1994; Slack, 1994). A modification of the original culture conditions by including retinoic acid along with activin permits however efficient and exclusive induction of pronephric tubules in vitro (Moriya et al., 1993). Histological analysis indicates that the induced pronephric tubules are identical to those observed in normal pronephroi (Uochi and Asashima, 1996). Furthermore, in vitro development of pronephric tubules parallels normal development at the molecular level as illustrated by the analysis of marker genes (Uochi and Asashima, 1996; Uochi et al., 1997). These findings indicate that retinoic acid plays an important role in early kidney development and establishes animal cap cultures as a complementary experimental system to study early events of pronephric kidney organogenesis.

**Pronephric kidney-derived cell lines**

From whole animal models over organ culture systems, cell culture models represent the next level of resolution in the analysis of the mechanisms underlying organogenesis. Cultures of renal cell lines, such as MDCK cells derived from the metanephric collecting-duct epithelium (Gaush et al., 1966) or A6 cells isolated from adult Xenopus mesonephric kidneys (Rafferty, 1969), have been successfully used to explore the establishment of epithelial cell polarity, the assembly of cell-cell junctions, and the regulation of renal solute transport (Duchatelle et al., 1992; Stevenson and Keon, 1998; Yeaman et al., 1999). It is believed that the mechanisms underlying these processes occur in a similar manner during normal kidney tubulogenesis. MDCK cells grown as cysts in collagen gels have also permitted the reconstitution of some aspects of tubule morphogenesis. Using such in vitro morphogenesis assays, Montesanto and colleagues were able to identify HGF as an inducer of epithelial tubulogenesis (Montesano et al., 1991a,b). Oversimplification is however a major risk associated when extrapolating results obtained from cell culture studies to in vivo kidney development. This is illustrated by findings that genetically engineered mice lacking HGF or its receptor, c-Met, appear to have grossly normal kidney development (Bladt et al., 1995; Schmidt et al., 1995; Uehara et al., 1995).

No Xenopus pronephric cell lines have been reported to date, but some have been isolated from other amphibian species. The WMHCA cell line is derived from primary explants of pronephroi obtained from embryonic stages of Rana pipiens and its appearance is predominantly epithelial (Wong and Tweedell, 1974). Several pronephric kidney tumor cell lines have also been isolated from explants of pronephric carcinomas (Tweedell and Wong, 1974). Pronephric cell lines have been used primarily to assay and propagate Lucké herpesviruses associated with the induction of renal adenocarcinomas (McKinnell, 1994). They, however, still remain poorly characterized at the cellular and molecular level. We found that WMHCA cells cannot be stained with monoclonal antibodies 3G8 and 4A6, directed against pronephric antigens (Vize et al., 1995). This suggests that WMHCA cells may have lost some characteristic features of differentiated pronephric epithelia in vivo (A.W. Brändli, unpublished results). Further studies will, however, be necessary to assess in full the potential uses of these cell lines as tools to investigate specific aspects of pronephric kidney development in vitro.

**Specification of the pronephric primordia**

The prospective pronephric area can be traced back in amphibians to the early gastrula stages. By means of vital staining, the area can be localized to the marginal zone ventrolateral to the blastopore (Pasteels, 1942). This material will give rise to the
intermediate mesoderm. The signals that direct patterning of the mesoderm towards pronephric lineages are unknown. The patterning of mesoderm is thought to be the result of opposing signals emitted by Spemann's organizer and the ventral side of the embryo (Graff, 1997; Heasmann, 1997; Stennard et al., 1997). This leads to the development of mesodermal tissues and organs, such as notochord, somites, pronephros, mesenchyme, and blood. Signaling factors, such as BMP and activin-like TGF-β family members, are involved in directing ventral and dorsal mesoderm formation, but none of the various factors alone is capable of instructing mesoderm to form pronephric tissue (Moriya et al., 1993; Dosch et al., 1997). As mentioned above, only activin in combination with retinoic acid can promote pronephric cell fates in explant cultures (Moriya et al., 1993; Uochi and Asashima, 1996). Whether retinoic acid cooperates with activin or a related TGF-β family member to establish pronephric cell fate in vivo remains to be established.

The patterning of mesoderm occurs during gastrulation, and the specification of pronephric cell fate is a result of this process. Experiments done in urodeles indicate that the prospective pronephric area is capable of self-differentiation by midneurula stages (Fales, 1935). The timing of specification of pronephric tubules and duct in Xenopus embryos was recently examined in greater detail using molecular markers (Brennan et al., 1998). The authors found that pronephric tubules are specified by stage 12.5 in the pronephric anlagen whereas pronephric duct is specified later at stage 14. This is earlier than was previously accepted for urodele amphibia (Fales, 1935). The time point of pronephric capsule specification still remains to be elucidated. Early markers specific to the pronephric capsule, such as WT-1 (Carroll and Vize, 1996; Semba et al., 1996), might serve useful towards this goal.

Cell lineages of the intermediate mesoderm

The fully differentiated mature metanephric kidney is a complex structure composed of identical functional units, the nephrons. Each unit comprises at least 12 different epithelial cell types (Burkitt et al., 1993) with collecting duct epithelia deriving from the ureteric bud epithelium and the metanephrogenic mesenchyme generating the other cell types (Saxén, 1987; Ekblom, 1992; Davies and Bard, 1998). The metanephric mesenchyme forms also renal stroma, cells of the juxtaglomerular apparatus, and the vascular endothelium. Finally, the neural crest is thought to be the source of cells with neuronal properties that populate the metanephric kidney (Davies and Bard, 1998). The diversity of cell types found in the mature kidney...
Drummond, 1999). It can therefore be expected that Xenopus may represent glomerular mesangial cells (Majumdar and smooth muscle cells are present in the zebrafish pronephros and cells. Several lines of evidence suggest the existence of a common bipotential precursor for these cell types, the hemangioblast (Risau and Flamme, 1995; Risau, 1997; Choi et al., 1998; Gering et al., 1998). Remarkably, overexpression of the bHLH transcription factor SCL in zebrafish embryos results in excessive production of hematopoietic and endothelial precursors at the expense of somitic and pronephric tissues (Gering et al., 1998). SCL expression appears therefore to mark the hemangioblast lineage. SCL+ cells, possibly hemangioblasts, are found in Xenopus adjacent to the pronephros and the pronephric duct (Mead et al., 1998). An open question is whether both splanchic and somatic layers of the intermediate mesoderm. The pronephric kidney with its simple structural organization may represent a more convenient system to study molecular mechanisms underlying the generation of cellular diversity in the developing kidney. Many, but not all cell types can now be identified with the help of specific marker genes (Table 1). This should allow in the future the dissection of the cell lineage relationships in the intermediate mesoderm. Following components will be necessary to assemble a functional pronephric kidney: blood vessels, a pronephric capsule, and tubular and duct epithelia. Remarkably, all these components arise from a single intermediate mesoderm. A model depicting the inferred lineage relationships in the intermediate mesoderm is shown in Figure 2. The pronephric anlage is derived from the somatic layer of the intermediate mesoderm. It will generate tubule and duct primordia, which will differentiate to form the epithelia lining the pronephric tubules and duct, respectively. The splanchic layer of the intermediate mesoderm gives rise to the anlage of the pronephric capsule, which will generate visceral and parietal epithelia.

While much attention has been paid to development of the duct and tubular epithelia of the pronephric kidney, little is known about interstitium and the cell types forming the glomus in Xenopus. Interstitial or stromal cells usually surround renal epithelia. An exception is the glomerulus, where the epithelia are directly adjacent to endothelial cells. Pronephric interstitial cells have not been described to date. It would however be surprising if pronephric kidneys were to lack a pathway that generates interstitial cell lineages. In the mature metanephric kidney, only 6% of the space is occupied by interstitial cells (Ekbom and Weller, 1991). Low abundance in comparison to tubular epithelia may explain why pronephric interstitial cells might have so far escaped detection. Currently, no molecular markers for interstitial cells in Xenopus are known. The winged-helix transcription factor BF-2 might however be a promising candidate gene, as it is essential for the development of stromal cell lineages in the metanephric kidney (Hatini et al., 1996). Cells with morphology similar to aortic pericytes or smooth muscle cells are present in the zebrafish pronephros and may represent glomerular mesangial cells (Majumdar and Drummond, 1999). It can therefore be expected that *Xenopus* pronephroi harbor mesangial cells too. The development of metanephric glomerular pericytes and mesangial cells is dependent on the growth factor PDGF-B and its receptor PDGFRβ (Levéen et al., 1994; Soriano, 1994). These cell types might therefore be detected in *Xenopus* embryos with reagents (e.g. antibodies) directed against PDGFRβ.

The juxtaglomerular apparatus is involved in the regulation of blood pressure via the renin-angiotensin-aldosterone mechanism. It is made up of three components, the macula densa, juxtaglomerular cells, and the extraglomerular cells (Burkitt et al., 1993). The macula densa is a specialized area of the distal metanephric tubule, that acts as a sodium sensor. The juxtaglomerular cells are specialized smooth muscle cells of the glomerulus that secrete the enzyme renin. The function of the extraglomerular mesangial cells, the third cell type of the juxtaglomerular apparatus remains obscure. In *Xenopus*, renin-positive cells do apparently not appear in the pronephros, but are detected in the walls of afferent arterioles within the mesonephros (Tahara et al., 1993). These findings indicate that the pronephric kidney may lack juxtaglomerular cells. Expression of amiloride-sensitive epithelial sodium channels (ENaC) in pronephric epithelia (A. Terrettaz and A.W. Brändli, unpublished observations; see below) suggests, however, that some aspects of sodium homeostasis and thus of the renin-angiotensin-aldosterone regulatory system are established already at the level of the pronephros. Further studies will be necessary to determine which components of the blood pressure control system are present in the pronephric kidney.

Intermediate and lateral plate mesoderm is also the source of vascular endothelial (angioblasts) and hematopoietic precursor cells. Several lines of evidence suggest the existence of a common bipotential precursor for these cell types, the hemangioblast (Risau and Flamme, 1995; Risau, 1997; Choi et al., 1998; Gering et al., 1998). Remarkably, overexpression of the bHLH transcription factor SCL in zebrafish embryos results in excessive production of hematopoietic and endothelial precursors at the expense of somitic and pronephric tissues (Gering et al., 1998). SCL expression appears therefore to mark the hemangioblast lineage. SCL+ cells, possibly hemangioblasts, are found in *Xenopus* adjacent to the pronephros and the pronephric duct (Mead et al., 1998). An open question is whether both splanchic and somatic layers of the intermediate mesoderm contain hemangioblastic cell populations. Recent findings suggest the existence of two distinct lineages, a somatic lineage strictly endothelial and a splanchic lineage with dual potential for angiopoiesis and hematopoiesis (Pardanaud et al., 1996).
Blood vessels are constructed during embryogenesis by two processes: vasculogenesis, whereby a primitive vascular network is established from endothelial progenitors, and angiogenesis, in which preexisting vessels send out capillary sprouts to produce new vessels (Risau and Flamme, 1995; Folkman and D’Amore, 1996; Risau, 1997). Both processes are employed to generate the vasculature of the pronephric kidney in Xenopus. From stage 24 onward, precursor cells expressing the endothelial markers Flk-1 and Msr are evident in lateral stripes that extend from the future pronephric sinus towards the cloaca (Devic et al., 1996; Cleaver et al., 1997). These cells will differentiate by a vasculogenic mechanism to form the posterior cardinal vein and the pronephric sinus permeating the space between the pronephric tubules (Cleaver and Krieg, 1998). On the other hand, sprouting angiogenesis from the dorsal aorta most likely generates the arteries of the glomus. Interestingly, the dorsal intermediate mesoderm harbors a bipotential pool of Flk-1 expressing angioblasts (Cleaver and Krieg, 1998). This pool of precursor cells will generate migratory angioblasts that will go on to form the dorsal aorta, and stationary angioblasts that will develop the venous vasculature of the pronephros.

There are two sites of hematopoiesis in the Xenopus embryo, the ventral blood islands, and the dorsal lateral plate region (Zon, 1995; Huber and Zon, 1998). The latter region is located near the pronephric tubules and duct. It is analogous to the AGM region identified as an intraembryonic site of hematopoiesis in other vertebrates (Dzierzak and Medvinsky, 1995). The hematopoietic stem cells of the dorsal lateral plate give rise to the definitive lineages, which will ultimately colonize the fetal liver and thymus. The hematopoietic stem cells in the dorsal and ventral regions have a common origin in the ventrolateral mesoderm of the gastrula and become committed to hematopoiesis during neurula stages (Turpen et al., 1997). Remarkably, the timing of commitment coincides with the specification of pronephric tubule and duct primordia (Brennan et al., 1998), indicating that major cell lineage decisions in the intermediate mesoderm occur concomitantly in the neurula embryo. The zinc finger transcription factor GATA-2 is required for the maintenance or proliferation of hematopoietic progenitors (Tsai et al., 1994). In Xenopus, GATA-2 expression is found in the pronephric region of stage 28 embryos (Kelley et al., 1994; Bertewistle et al., 1996; Turpen et al., 1997). This suggests that the initiation of the dorsal (definitive) hematopoietic program occurs in the late tailbud stages. The appearance and differentiation of hematopoietic derivatives in the region of the pronephric kidney have been studied in different frog species (Carpenter and Turpen, 1979; Turpen and Knudson, 1982; Frank, 1988). These studies show that granulopoiesis is the predominant hematopoietic activity in the area of pronephric tubules and in the mesenchymal sheets surrounding the pronephric duct. Erythropoiesis accounts for a minor component of the hematopoietic activity, and lymphopoiesis within the organ is negligible. These findings have recently been largely confirmed using molecular markers (Turpen et al., 1997). Taken together, this suggests that simultaneously with the induction of tissues, such as vasculature and pronephric epithelia, in the intermediate mesoderm, some cells are maintained as hematopoietic stem cells that will later contribute to definitive hematopoiesis.

The adrenal gland develops in close association with the metanephric kidney from cells of dual embryonic origin (Carlson, 1996). The adrenal cortex originates from the intermediate mesoderm and contains adrenocortical cells that control kidney function by secreting aldosterone and other steroid hormones. The medulla of the adrenal gland arises from migrating trunk neural crest cells. Chromaffin cells, an important adenomedullary cell type, are active in the production of neurotransmitters, such as epinephrine and norepinephrine. Very little is known about the development of the adrenal gland in relation to the pronephric kidney. The interrenal tissue of the adrenal glands (homologous with the mammalian adrenal cortex) becomes morphologically detectable in the dorsal mesentery of stage 42 and 43 Xenopus embryos (Nieuwkoop and Faber, 1994). Neural crest-derived cells, potential chromaffin cells, have been mapped to the pronephric duct, the posterior cardinal veins and its branches into the pronephric kidney (Krotocki et al., 1988; Callazo et al., 1993). Adrenal gland components appear therefore likely to be embedded in the pronephric kidney around the posterior cardinal vein and its derivatives. The morphology, histology, and precise anatomical relationship of the adrenocortical and adenomedullary homologs to the Xenopus pronephric system remain to be determined. This will, however, require the establishment of adequate markers for adrenal cells in Xenopus.

**Molecular anatomy of the pronephric kidney**

The cascades of molecular events that determine the different cell lineages of the intermediate mesoderm and drive pronephric kidney development are largely unknown. This is illustrated by the observation that of the dozen or more mutant mouse strains with kidney developmental defects (Davies and Brändli, 1997; Davies and Bard, 1998) only two genes have been identified to date that are essential for early kidney development. *Lim-1* encodes a LIM class homeodomain, which may be essential for the development of the entire urogenital system (Shawlot and Behringer, 1995), and the paired-box transcription factor Pax-2 is necessary for elongation and/or maintenance of the nephric duct (Torres et al., 1995; Favor et al., 1996). No other genes have been demonstrated to be essential for the development of the pronephric kidney.

It is obvious that the induction of pronephric development and the subsequent profound morphological changes associated with this process must be accompanied by changes in gene expression patterns including various types of molecules: growth factors, receptors, intracellular signaling molecules, extracellular matrix constituents and cell adhesion molecules. Identifying these genes and defining their precise spatial and temporal sequence of expression from the specification of pronephric lineages to the mature, functional pronephric kidney represents therefore an important step towards understanding kidney organogenesis. A few years ago, I began with my collaborators a systematic screen for genes activated during pronephric kidney development in Xenopus. Faced with limited resources, we concentrated our efforts initially on the known Xenopus genes, which are at present well over 2000 unique genes. Many of these genes encode developmental control genes and our colleagues in the Xenopus community had accumulated a wealth of information on their early embryonic expression. The main focus of research activities in Xenopus had traditionally been on problems associated with embryonic axis specification, induction and patterning of mesoderm, and early neurogenesis. The temporal and spatial gene expression patterns were therefore usually only documented until late neurula stages.
For several genes, however, pronephric expression had been reported. These include the transcription factors Emx-1 (Pannese et al., 1997), ets-2 (Meyer et al., 1997), HNF-1β (LFB-3) (Demartis et al., 1994), Iro-3 (Bellefroid et al., 1998), Lim-1 (Taira et al., 1994), Pou-2 (Witta et al., 1995), Sal-1 (Hollemann et al., 1996), WT-1 (Carroll and Vize, 1996; Sembba et al., 1996), and XFD-11 (Koster et al., 1998); the secreted factors BMP-7 (Wang et al., 1997), gremlin (Hsu et al., 1998), VEGF (Cleaver et al., 1997), WIF-1 (Hsieh et al., 1999), and Wnt-4 (D. Saulnier and A.W. Brändli, manuscript in preparation); the cell surface receptors integrin-α6 (Lallier et al., 1996) and frizzled-3 (Shi et al., 1998); and the Na⁺, K⁺ ATPase pump (Uochi et al., 1997). Interestingly, some of these genes have been shown to be essential for metanephric kidney development in the mouse, such as Wnt-4 (Stark et al., 1994), BMP-7 (Dudley et al., 1995; Luo et al., 1995; Jena et al., 1997), and WT-1 (Kreidberg et al., 1993). Other genes, such as Sal-1, are orthologs of human genes implicated in syndromes manifested by renal abnormalities (Kohlhase et al., 1998). It therefore appears that some of the molecular players necessary for normal metanephric kidney development may have a role in the developing pronephric kidney. A few notable examples (WT-1, HNF-1β, and ets-2) apart, the pronephric expression patterns of the above-mentioned genes were at best incompletely documented. Particularly, onset and duration as well as tissue specificity of pronephric gene expression was unknown. Towards this goal, probes were obtained to establish by in situ hybridization and serial sectioning each gene’s specific pronephric expression profile (S. Eid, H. Ghanbari, N. Heller, D. Saulnier, A. Terrettaz, and A.W. Brändli; unpublished results). A second strategy was based on conclusions drawn from results emerging from the various ongoing and completed genome projects. It appears that evolution has only provided for a limited number of highly conserved signaling pathways to control developmental processes in animals ranging from C. elegans to vertebrates (Ruvkun and Hobert, 1998). Thus, rather than inventing novel pathways for every newly evolving body structure, existing ones would be reused in a variety of novel manners and combinations. Repeated whole genome duplications as those postulated to have occurred early in vertebrate evolution (Sidow, 1996), might have relieved certain constraints intrinsic to this strategy. Signal transduction pathways controlled by the Notch, hedgehog, FGF, BMP and Wnt gene families constitute important signaling pathways controlling vertebrate development. We therefore systematically screened Xenopus embryos for pronephric expression of gene products associated with the above-mentioned signal transduction pathways. In a third strategy, finally, we cloned Xenopus orthologs of mammalian genes implicated in the control of early kidney development, such as Pax-2 and Pax-8 (Heller and Brändli, 1997, 1999). Similar screening projects are currently being carried out by Vize and colleagues (Carroll et al., 1999b).

What have we learnt since initiating the pronephric marker gene project? To date, well over 200 genes were examined by in situ hybridization and about 30 genes (including the genes mentioned above) were found or confirmed to have specific expression during growth and development of the pronephric kidney. A regularly updated catalog which combines our data on gene expression patterns with information taken from the literature can be found in the pro- and mesonephros section of the Kidney Development Database (Davies and Brändli, 1997). Selected examples of pronephric gene expression in tadpole staged embryos are shown in Figure 3, and the temporal succession of marker gene expression with respect to the three compartments of the pronephric kidney are given in Figure 4. Most of the Xenopus genes found to be expressed during pronephric development have mammalian counterparts that are involved in the development of the metanephric kidney. Expression occurs in structures homologous to the two kidneys. For example, Wnt-4 expression is associated with tubulogenesis in the pronephric tubule anlage (see Fig. 3D) as well as in the metanephrogenic blastema (Stark et al., 1994; Kispert et al., 1996). Coexpression of the Wnt receptor frizzled-3 with Wnt-4 suggests that they may form a signaling complex in vivo (D. Saulnier and A.W. Brändli; manuscript in preparation). Notch signaling is an evolutionary conserved mechanism that is used by multicellular organisms to control cell fates through local cell

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**Fig. 5. A model for pronephric kidney organogenesis in Xenopus laevis.** The schematic representation shows a lateral view of the intermediate mesoderm (anterior to the left, dorsal up) depicting the development of the tubular and duct components of the pronephric kidney. For simplicity, the pronephric corpuscle is not shown. Hallmarks of pronephric kidney development are given to the left, while relevant embryonic stages of Xenopus development are indicated on the right. Abbreviations: D, pronephric duct; DA, duct anlage; NM, nephrogenic mesenchyme; PA, pronephric anlage; RD, rectal diverticulum; T, pronephric tubules; TA, tubule anlage.
interactions (Artavanis-Tsakonas et al., 1999). Our studies have provided evidence for expression of the Notch ligand Delta-1 (Henrique et al., 1995) in the pronephric tubule anlage (Fig. 3C; S. Eid and A.W. Brändli, unpublished results). This finding identifies the pronephros as a novel organ where Notch-mediated cell fate control may act. Tissue-specific bHLH proteins (e.g. MyoD, NeuroD) are important transcription factors controlling cell fate and differentiation in muscle, neurons, and many other tissues (Murre et al., 1994). Xenopus Id-2 (Wilson and Mohun, 1995; Gawantka et al., 1998), a member of the inhibitory HLH protein family that can bind tissue-specific bHLH proteins (Norton et al., 1998), was expressed in the developing pronephric duct (S. Eid and A.W. Brändli, unpublished results). This provides indirect evidence for the existence of tissue-specific basic HLH proteins that function during pronephric kidney differentiation. Indeed, we have recently succeeded in isolating cDNAs encoding tissue-specific bHLH class proteins from a Xenopus kidney cDNA library (S. Eid and A.W. Brändli, unpublished results). Collectively, these observations establish a promising basis for more substantial studies aimed at addressing the relative contributions of these signaling cascades to pronephric kidney organogenesis.

A comparison of the temporal and spatial profiles of gene expression in developing pronephros revealed at least five discrete periods where the expression of new sets of gene products is initiated (Fig. 4). Interestingly, these time points coincide with major developmental events, such as the onset of organogenesis. Furthermore, the observed spatial patterns led to a more refined definition of the developmental anatomy of the pronephric kidney. Expression of the earliest markers of the future pronephric kidney (HNF-1β, Lim-1 and Pax-8) was detected during late gastrulation at stages 12/13 (Demartis et al., 1994; Taira et al., 1994; Heller and Brändli, 1999). This corresponds precisely with the time when specification of pronephric tubule and duct lineages occurs (Brennan et al., 1998). Remarkably, expression of all three genes in the intermediate mesoderm is confined to an area comprising the prospective tubule and duct primordia. Serial sections show that the expression of HNF-1β is restricted to the somatic layer of the intermediate mesoderm (Demartis et al., 1994). At present, it is not known whether Pax-8 and Lim-1 are similarly restricted to the somatic intermediate mesoderm only. These findings, however, suggest that at least tubule and duct compartments of the future pronephric kidney arise from a common pronephric anlage, which is established in the late gastrula embryo. HNF-1β, Lim-1 and Pax-8 may therefore constitute part of the molecular machinery controlling specification of the pronephric anlage. The complete absence of renal tissue in Lim-1 deficient mice (Shawlot and Behringer, 1995) strongly supports this notion.

Pronephric kidney morphogenesis begins at stage 20/21 with the thickening and condensation of both tubular and duct primordia (Nieuwkoop and Faber, 1994; Vize et al., 1997). This coincides with the onset of differential gene expression in the common pronephric anlage (Fig. 4). The antero-dorsal region of the pronephric anlage, fated to develop pronephric tubules (Vize et al., 1995), initiates expression of Wnt-4 and frizzled-3, possible inducers or modulators of tubulogenesis, and Delta-1, an indicator of ongoing cell fate selection. Wnt-4, frizzled-3 and Delta-1 are therefore the earliest markers for the pronephric tubule anlage. On the other hand, the ventro-posterior region of the pronephric primordium, which is destined to form the pronephric duct (Vize et al., 1995), expresses the POU-class transcription factor Pou-2 (D. Saulnier and A.W. Brändli, unpublished results). Furthermore, the pronephric capsule anlage emerges as a molecularly distinct compartment by initiating WT-1 expression (Carroll and Vize, 1996; Semba et al., 1996). WT-1 was recently shown to be required for the development of glomerular podocytes in the mouse metanephros (Moore et al., 1999), and thus may have a similar role in the pronephros. Interestingly, Pax-2 expression occurs in tubule as well as duct primordia (Fig. 3B; Heller and Brändli, 1997). This could indicate that Pax-2 controls a developmental process, such the transition from mesenchyme to epithelia, occurring simultaneously in both primordia. The slightly later expressed ets domain transcription factor ets-2 (Meyer et al., 1997) shares an expression pattern largely overlapping with Pax-2 and might therefore serve a similar function. The beginning segregation of pronephric tubules and the onset of pronephric duct extension characterize midblastula stages (Nieuwkoop and Faber, 1994). Expression of integrin-αβ in the pronephric duct is initiated at stage 26 (Lallier et al., 1996). Given its function as an extracellular matrix receptor, it is conceivable that integrin-αβ participates in some aspect of pronephric duct cell migration. The expression of the homeobox transcription factor Iro-3 and the zinc-finger transcription factor Sal-1 in the developing pronephric tubules and duct epithelia, respectively, suggests roles in the patterning of these epithelia (Fig. 3E,F). Interestingly, a fish homolog of Xenopus Sal-1, medaka spalt, has been recently postulated to act as a target gene of hedgehog signaling (Köster et al., 1997). The expression of Sal-1 might therefore provide indirect evidence for a role of hedgehog signaling during pronephric kidney development. Two more waves of gene expression, around stages 30 and 37/38 can be distinguished. These are mainly associated with the maturation and terminal differentiation of the pronephric epithelia and will be discussed below in greater detail. Expression of VEGF in the pronephric capsule compartment is however noteworthy here (Cleaver et al., 1997). VEGF acts as a chemottractant for endothelial cells (Cleaver and Krieg, 1998), and thus might function as a morphogen for dorsal aorta-derived endothelial capillaries that will form the pronephric glomus. The initiation of VEGF expression defines therefore an important step in the maturation of the pronephric capsule.

Functional differentiation of pronephric epithelia

Once the basic architecture of the Xenopus pronephros has been established in the late tailbud embryo (st. 29/30), pronephric tubules and ducts continue to mature. This requires the formation of luminal spaces, cell proliferation within existing tubular epithelia, and functional maturation. Two recently described monoclonal antibodies recognize antigens that are tissue-specific markers of the differentiating pronephric kidney (Vize et al., 1995). Antibody 3G8 stains the apical membrane of pronephric tubules from stage 31 on, whereas antibody 4A6 recognizes a 50 kDa protein that localizes to both cell surfaces of duct epithelia from stage 38 onward. The precise identity and nature of the epitopes are unknown, but their subcellular distributions suggest that they are either components of the plasma membrane or the extracellular matrix.

Pronephric kidney maturation will also require the appearance of specialized ion channels and transporters on the plasma membrane.
of pronephric epithelia. These maturation processes are essential for the formation of functionally active pronephric kidneys. At present, they are however poorly understood. The Na+, K+ ATPase pump located in the basolateral membrane of renal epithelia, drives most of the renal transepithelial transport by producing an electrochemical gradient between the intra- and extracellular spaces across the cell membrane (Geering, 1997). Active transport of sodium is associated with secondary active and passive reabsorption of many other solutes as well as the bulk of water. The pronephric expression pattern of the α subunit of the Xenopus Na+, K+ ATPase was recently reported (Uochi et al., 1997). Remarkably, the expression of this pump in tubular and duct epithelia begins at around stage 30 and thus coincides with the onset of pronephric kidney maturation. The establishment of osmoregulatory and reabsorptive functions in the pronephric kidney will, however, require the expression of a multitude of additional genes encoding transporters, channels, and pumps, which have largely not been identified yet in Xenopus or any other lower vertebrate. Over the last years, Xenopus cDNAs for cystic fibrosis transmembrane regulator (CFTR), the renal chloride channel CIC-5, and several subunits of the epithelial amiloride-sensitive sodium channel ENaC have been isolated (Tucker et al., 1992; Puoti et al., 1995, 1997; Lindenthal et al., 1997; Price et al., 1996). It is currently unclear which of these candidate genes become indeed activated in the developing pronephric kidney. Our own analysis has indicated expression of ENaC subunits in the distal common tubule and the pronephric duct from stage 32 onwards (A. Terrettaz and A. W. Brändli, unpublished observations). These preliminary findings strongly suggest that pronephroi are capable of sodium homeostasis. Furthermore, they indicate that renal ENaC expression in terminal nephron segments has been conserved between pronephric and metanephric kidneys. Similar mechanisms may therefore exist that establish proximal-distal patterning along the length of a nephron during pronephric and metanephric kidney development.

A model for pronephric kidney organogenesis

The ongoing efforts to map the spatial distribution of pronephric gene expression as a function of developmental stage have provided a wealth of information. Although far from complete, for example serial sections will be necessary to more accurately define the domains of gene expression within the pronephric kidney, the emerging results ask for a revision of our traditional understanding of pronephric kidney development. Novel molecular markers will facilitate the description of the initial appearance of the various cell lineages generated by the intermediate mesoderm. The cellular diversity of the pronephric tubular and duct epithelia is poorly understood. Distinct segments of the pronephric tubular system can now be distinguished by staining with specific molecular markers (Fig. 3). Further studies will be necessary to extend the emerging molecular anatomy of the pronephric kidney to the histological and ultrastructural level. Finally, the relationship of the successive vertebrate kidneys has become clearer with the discovery that many of the same regulatory molecules, e.g. Wnt-4 and BMP-7, are involved in the development of each kidney. It is however currently too early to make assumption on the functions of the different marker genes during pronephric kidney development. Many of the suggestions given above are arguable and therefore highly speculative. Nevertheless, they serve as a basis for future experimentation.

The recent work in Xenopus reviewed here suggests the following revised model for the events involved in pronephric kidney development (Fig. 5). During blastula stages, mesoderm is induced by FGF- and activin-like factors. While gastrulation occurs, mesoderm is patterned to by unknown signals to form intermediate mesoderm, which will give rise to the excretory system, the vasculature, and the hematopoietic system. With the completion of gastrulation, the pronephric anlage becomes specified in the anterior intermediate mesoderm presumably by the activity of the HNF-1β, Lim-1 and Pax-8 genes. The posterior intermediate mesoderm is set aside as nephrogenic mesenchyme that will later give rise to mesonephric tubules. During neurula stages, the pronephric anlage will be patterned to form the tubule, duct, and capsule primordia. It is not known how many steps and what factors are necessary for this process. During tadpole stages, growth, morphogenesis, and cellular diversification of the pronephric kidney occurs. This may involve the activity of Wnt, BMP, hedgehog, and Notch signaling cascades. Functional maturation begins with the expression of the Na+, K+ ATPase in late tailbud stage embryos and proceeds concomitantly with ongoing morphogenesis. Two events, the fusion of pronephric duct and rectal diverticulum and onset of blood circulation, mark the completion of pronephric kidney organogenesis.

Conclusions and perspectives

The mammalian metanephric kidney and the Xenopus pronephric kidney represent two specialized solutions of the same problem, urine formation. Despite differences with respect to complexity, both kidneys share common features with regard to morphology and structural organization. Emerging evidence suggests that this is likely to extend to key aspects of renal physiology. The Xenopus pronephric kidney is therefore an attractive model to study kidney organogenesis and the establishment of renal function. Significant progress has been made during the last years in establishing this model. An in vitro organ culture system recapitulating pronephric tubule induction has been developed (Moriya et al., 1993; Uochi and Asashima, 1996), the timing of specification of the major pronephric lineages has been determined (Brennan et al., 1998), and several key genes controlling kidney development have been cloned (Carroll and Vize, 1996; Semba et al., 1996; Heller and Brändli, 1997, 1999). Our knowledge of pronephric kidney development is however still rudimentary. Limited analysis of marker gene expression has revealed a surprising complexity of pattern within the developing pronephric kidney that had previously not been anticipated. Major unanswered questions in pronephric kidney development include:

- What is the nature of the signals that pattern mesoderm into intermediate mesoderm?
- How are the major derivatives of the intermediate mesoderm (angioblasts, hematopoietic stem cells, and pronephric cell lineages) generated?
- Are there pronephric stem cells capable of self-renewal and giving rise to a variety of pronephric cell types?
- What induces condensation and epithelialization of the pronephric anlage?
- What are the guidance cues controlling pronephric duct elongation?
- What is the molecular basis of nephron segmentation?
These issues are however not merely only relevant with respect to pronephric kidney development, but are of central importance for our understanding of the molecular and cellular events underlying vertebrate kidney development in general. The *Xenopus* system is anticipated to play a central role in addressing many of these questions in the coming future. There has been to date considerable success in characterizing genes that regulate mesoderm formation in *Xenopus* (Kessler and Melton, 1994; Graff, 1997; Heasmann, 1997; Stennard et al., 1997). In a similar manner, pronephric gene function may now be revealed by microinjecting cDNA and RNAs into embryos and careful observation of the effects on whole embryo development in general and pronephric kidney formation in particular. First studies addressing the function of *WT-1* (Wallingford et al., 1998) and *Pax-2* isoforms (N. Heller, H. Ghanbari and A.W. Brändli, manuscript in preparation) have been completed and have revealed intriguing findings with respect to the molecular control of *Xenopus* pronephric kidney development. *In vitro* and *in vivo* assays will have to be developed to determine the lineage potential of pronephric stem cells and progenitors. Towards this goal, explant cultures prepared from early embryos injected with particular genes may be employed to test for cell fate changes. Expression cloning strategies will be useful in the identification of novel factors involved in these events. Finally, large-scale gene expression screens (Gawantka et al., 1998) with randomly picked pronephric kidney cDNAs and expression analysis by whole-mount *in situ* hybridization should provide a multitude of novel marker genes and identify critical genes whose expression may be limited in time and space. Together, these experimental approaches aim to expand our knowledge of kidney development and may lead to a powerful novel paradigm for understanding how molecular and cellular events lead to organogenesis.

**Summary**

Kidney development is distinguished by the sequential formation of three structures of putatively equivalent function from the intermediate mesoderm, the pronephros, mesonephros, and metanephros. While these organs differ morphologically, their basic structural organization exhibits important similarities. The earliest form of the kidney, the pronephros, is the primary blood filtration and osmoregulatory organ of fish and amphibian larvae. Simple organization and rapid formation render the *Xenopus* pronephric kidney an ideal model for research on the molecular and cellular mechanisms dictating early kidney organogenesis. A prerequisite for this is the identification of genes critical for pronephric kidney development. This review describes the emerging framework of genes that act to establish the basic components of the pronephric kidney: the corpuscle, tubules, and the duct. Systematic analysis of marker gene expression, in temporal and spatial resolution, has begun to reveal the molecular anatomy underlying pronephric kidney development. Furthermore, the emerging evidence indicates extensive conservation of gene expression between pronephric and metanephric kidneys, underscoring the importance of the *Xenopus* pronephric kidney as a simple model for nephrogenesis. Given that *Xenopus* embryos allow for easy testing of gene function, the pathways that direct cell fate decisions in the intermediate mesoderm to make the diverse spectrum of cell types of the pronephric kidney may become unraveled in the future.

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