Int. J. Dev. Biol. 43: 381-395 (1999)

Towards a molecular anatomy of the *Xenopus* pronephric kidney

ANDRÉ W. BRÄNDLI*

Institute of Cell Biology, Swiss Federal Institute of Technology, Zürich, Switzerland

CONTENTS

Introduction **381**
Structural organization of the pronephric kidney **382**
Development of the pronephric kidney **383**
In vitro induction of pronephric tubules **384**
Pronephric kidney-derived cell lines **385**
Specification of the pronephric primordia **385**
Cell lineages of the intermediate mesoderm **386**
Molecular anatomy of the pronephric kidney **388**
Functional differentiation of pronephric epithelia **390**
A model for pronephric kidney organogenesis **391**
Conclusions and perspectives **391**
Summary **392**

KEY WORDS: *pronephric kidney, Xenopus laevis, organogenesis, molecular markers, cell lineages*

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

Abbreviations used in this paper: AGM, aorta, gonad and mesonephros region; bHLH, basic helix-loop-helix domain; BMP, bone morphogenetic protein; ENaC, epithelial sodium channel; FGF, fibroblast growth factor; GDNF, glial cell-line derived neurotrophic factor; GFR, GDNF family receptor; GPI, glycosyl-phosphatidylinositol; HGF, hepatocyte growth factor; HNF, hepatocyte nuclear factor; LIM, Lin-.11, Isl-1 and Mec-3 homeodomain protein; MDCK, Madin-Darby canine kidney; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; PI-PLC, phoaphatidylinositol-specific phospholipase C; POU, Pit-1, Oct1/2 and Unc-86 homeodomain protein; SCL, stem cell leukemia protein; TGF, transforming growth factor; WMPA, Wisconsin-Minnesota pronephros strain A; WT-1, Wilms' tumor suppressor gene 1; XFD, *Xenopus* fork head domain related protein.

***Address for reprints:** Institute of Cell Biology, Swiss Federal Institute of Technology, ETH-Hönggerberg, CH-8093 Zürich, Switzerland. FAX: +41-1-633 1069. e-mail: brandli@cell.biol.ethz.ch

0214-6282/99/$15.00
© UBC Press
Printed in Spain
www.ehu.es/ijdb

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, GFRα1) 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 lines 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 pronephroi of fish and amphibia typically contain 1-3 nephrons (using the number of renal tubules as the defining criteria), whereas metanephroi 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 “glomus (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

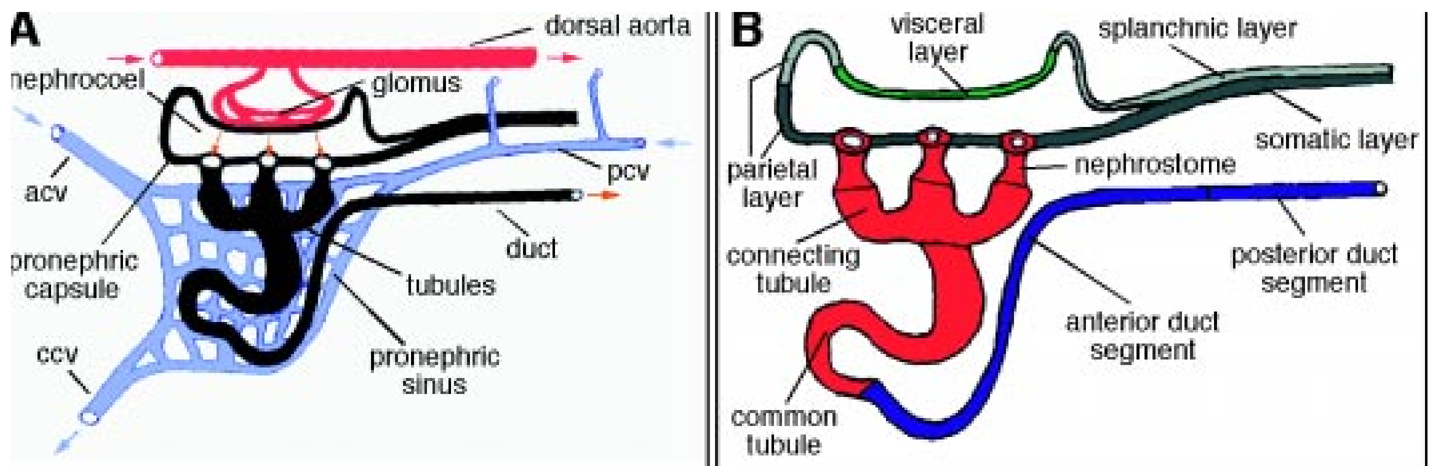

Fig. 1. Basic organization of the *Xenopus* pronephric kidney. (**A**) *Schematic representation of the Xenopus pronephric kidney. Blood vessels branching from the dorsal aorta form the glomus, the capillary network of the pronephric corpuscle. Blood travels from the dorsal aorta to the glomus where it undergoes size-selective pressure filtration. The plasma filtrate is collected in the nephrocoel and passes via ciliated nephrostomal funnels into the pronephric tubules. The tubular epithelia perform selective reabsorption of nutrients and salts. Blood from the posterior cardinal vein flows through the pronephric sinus, an ill-defined capillary network between the tubules, to return resorbed solutes to the circulatory system. The remaining excretory products pass into the pronephric duct, which empties into the cloaca.* (**B**) *Segmental organization of pronephric nephrons. The pronephric capsule is comprised of visceral and parietal epithelia. The visceral (or podocyte) layer (green) is a specialized segment of the splanchnic mesoderm. It contacts the vasculature of the glomus and is continuous with the epithelium of the parietal layer. Pronephric tubules (red) are subdivided into nephrostomes, connecting tubules, and the common tubule. Finally, the pronephric duct (blue) is comprised of at least two parts, the anterior and posterior duct segments. Abbreviations: acv, anterior cardinal vein; ccv, common cardinal vein; pcv, posterior cardinal vein.*

(Millard, 1949). Despite its simple architecture, the pronephric kidney contains all essential structural components necessary to function as a full-fledged excretory organ.

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). 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.

In vitro induction of pronephric tubules

The ability of metanephrogenic mesenchyme to develop in culture to metanephric kidneys has allowed the application of a variety of experimental procedures designed to investigate the properties of renal gene products, the activity of nephron-inducing agents, and the developmental mechanisms governing metanephric development (Saxén, 1987; Davies and Bard, 1998).

TABLE 1

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

Tissue type	Marker gene	References
Early pronephric anlage	*Pax-8*	Heller and Brändli, 1999
	HNF-1β	Demartis *et al.*, 1994
Tubule anlage and tubule epithelia only	*Wnt-4*	Mc Grew *et al.*, 1992; D.M.E. Saulnier and A.W. Brändli, manuscript in preparation
Duct anlage and duct epithelia only	*Pou-2*	Witta *et al.*, 1995; D.M.E. Saulnier and A.W. Brändli, manuscript in preparation
Tubule and duct epithelia, rectal diverticulum	*Pax-2*	Heller and Brändli, 1997
Rectal diverticulum only	*WIF-1*	Hsieh *et al.,* 1999
Pronephric capsule anlage and visceral (podocyte) epithelium	*WT-1*	Carroll and Vize, 1996; Semba *et al.*, 1996
Hemangioblasts, hematopoietic progenitors	*SCL*	Mead *et al.*, 1998
Angioblasts, endothelia	*Msr* *Flk-1*	Devic *et al.*, 1996 Cleaver *et al.*, 1997
Hematopoietic progenitors	*GATA-2*	Kelley *et al.*, 1994; Bertewistle *et al.*, 1996
Erythrocytes	*αT4 larval globin*	Walmsley *et al.*, 1994
Trunk neural crest cells	*PDGFRα*	Jones *et al.*, 1993; M. Mercola, personal communication

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 WMPA cell line is derived from primary explants of pronephroi obtained from embryonic stages of *Rana pipiens* and its appearance is predominantly epitheloid (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 WMPA cells cannot be stained with monoclonal antibodies 3G8 and 4A6, directed against pronephric antigens (Vize *et al.*, 1995). This suggests that WMPA 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*.

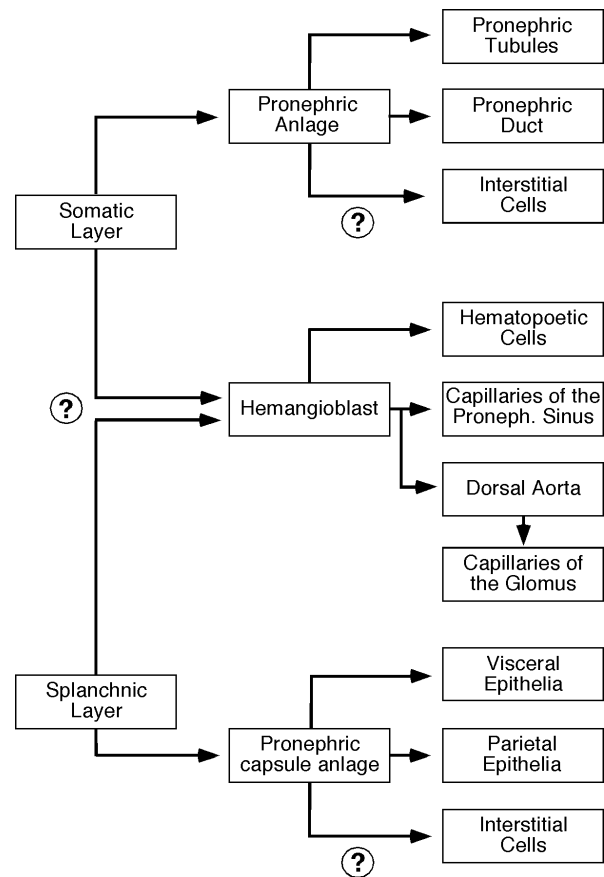

Fig. 2. Simplified scheme showing the relationship of intermediate mesoderm derivatives. *The intermediate mesoderm consists of the splanchnic layer facing the endoderm and the somatic layer contacting the ectoderm. The pronephric anlage develops from the somatic layer and generates the epithelia of pronephric tubules and duct. The splanchnic layer gives rise to the pronephric capsule anlage, which will differentiate to visceral (podocyte) and parietal epithelia. It is currently not known whether the pronephric capsule and/or pronephric anlagen generate interstitial cells. Intermediate and lateral plate mesoderm give also rise to hemangioblasts, the common precursors for hematopoietic and endothelial cells. It remains to be established whether both layers of the intermediate mesoderm generate hemangioblasts. Endothelial cells will contribute to the pronephric sinus and the vasculature of the glomus.*

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

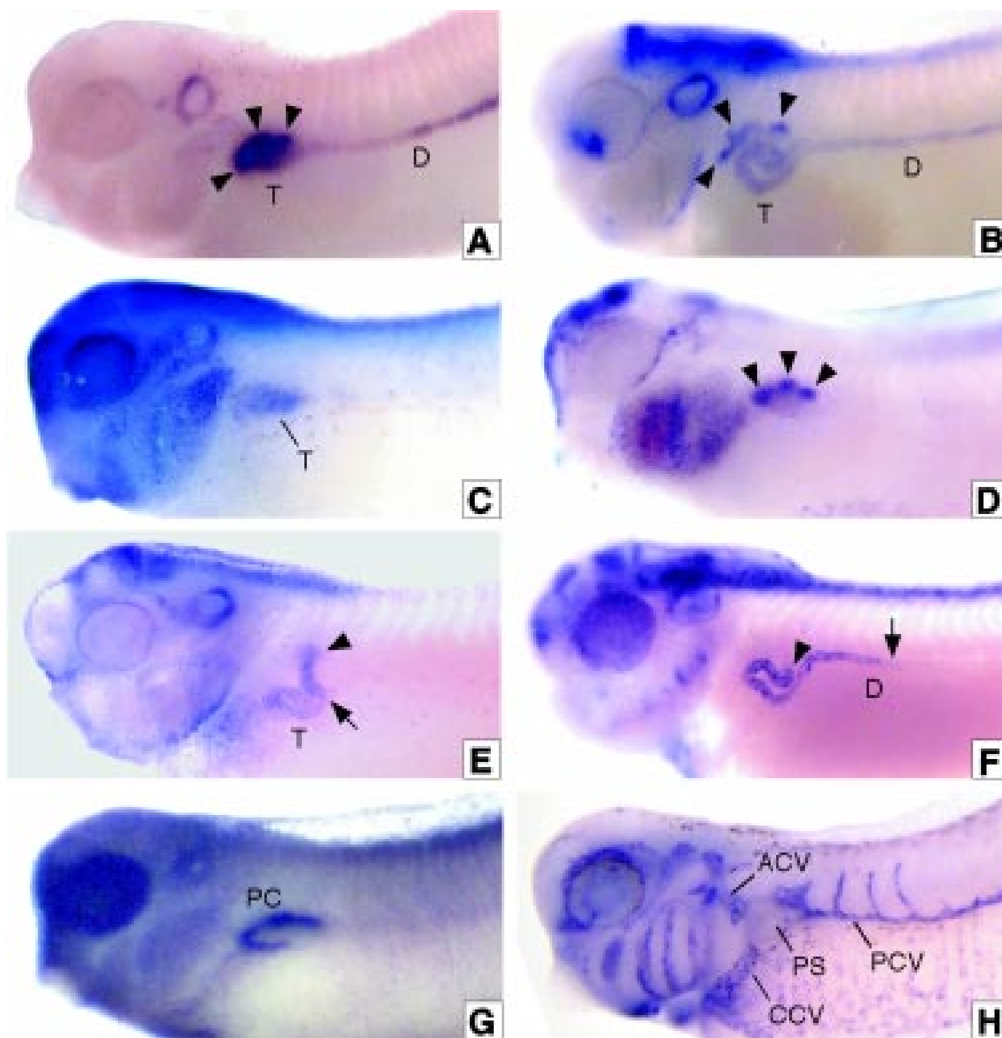

Fig. 3. Selected examples of marker gene expression in the developing *Xenopus* pronephric kidney. *Lateral views are shown anterior to the right. Transcripts were detected by whole-mount in situ hybridization.* **(A)** *Pax-8, stage 32. Expression is detected in the emerging pronephric tubules (arrowheads) and at reduced levels in the pronephric duct.* **(B)** *Pax-2, stage 36. All epithelia of the pronephric kidney including the nephrostomes (arrowheads) are stained.* **(C)** *Delta-1, stage 30. Staining is confined to the developing pronephric tubule anlage.* **(D)** *Wnt-4, stage 32. Expression is largely restricted to the nephrostomes (arrowheads).* **(E)** *Iro-3, stage 36. Transcripts are present in the common tubule only. Anterior (arrowhead) and posterior (arrow) ends of the expression domain are indicated.* **(F)** *Sal-1, stage 38. Expression is found in the common tubule (arrowhead) and the anterior segment of the pronephric duct (arrow indicates posterior end of the expression domain).* **(G)** *WT-1, stage 30. Staining is evident in the pronephric capsule.* **(H)** *Msr, stage 34. Staining marks the developing vasculature. The major blood vessels in the region of the pronephric kidney are indicated. In situ hybridization and photography was performed as described elsewhere (Heller and Brändli, 1997, 1999). Abbreviations: ACV, anterior cardinal vein; CCV, common cardinal vein; D, pronephric duct; PC, pronephric capsule; PCV, posterior cardinal vein; PS, pronephric sinus; T, pronephric tubules.*

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

appears therefore to constitute the final result of interactions between only four basic cell populations: epithelial cells of the ureteric bud, metanephrogenic mesenchyme, vascular endothelia, and primary neuroblasts. The details of the cell lineage relationships, the degree of pluripotency, and the sequences of cell fate choices taken in the developing metanephric kidney remain, however, still poorly understood.

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 source, the 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 splanchnic 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 (Ekblom 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

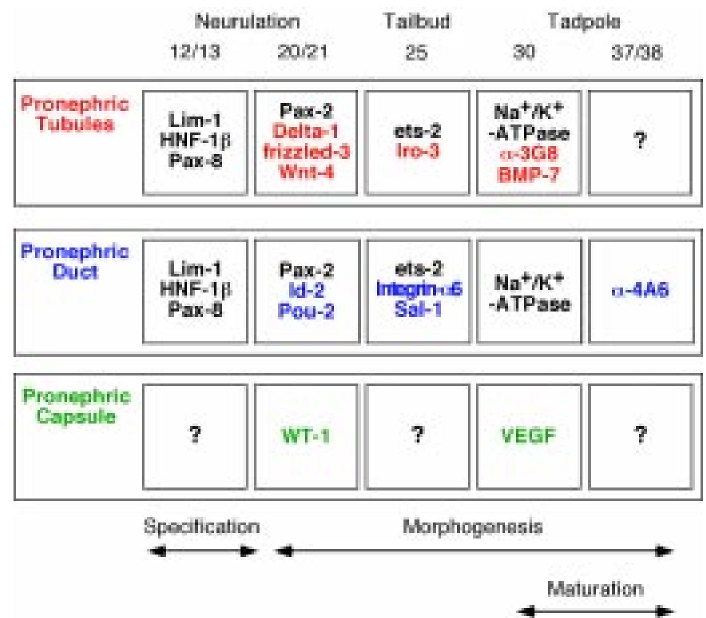

Fig. 4. Onset of gene expression within the pronephric kidney. *The temporal expression profile of marker genes was determined by whole-mount in situ hybridization. The onset of gene expression within the three compartments of the pronephric kidney is shown. Genes expressed exclusively in one compartment carry the same color code (tubules, red; duct, blue; capsule, green). Numbers refer to the embryonic stages of Xenopus laevis development.*

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 splanchnic 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 splanchnic 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 adrenomedullary 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 (Krotoski *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 adrenomedullary 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.

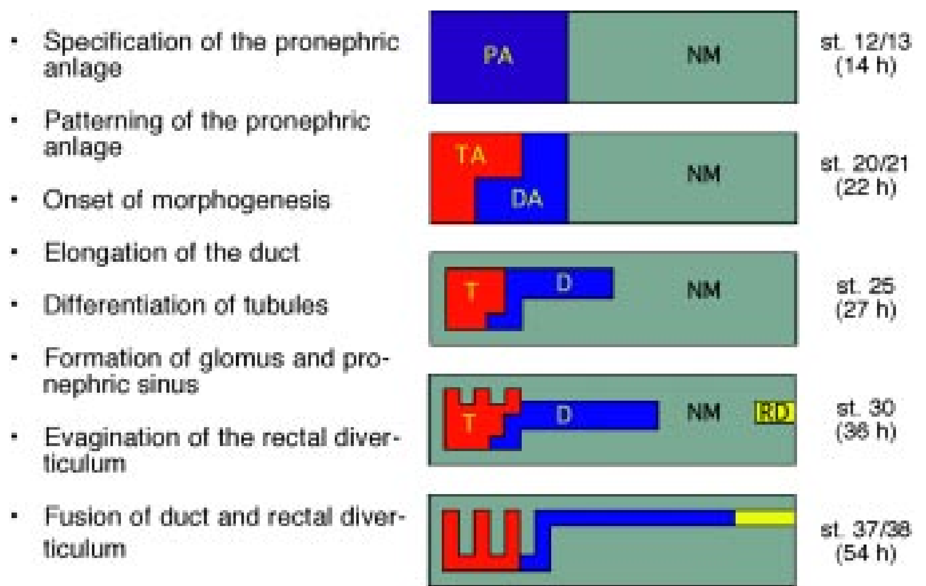

Fig. 5. A model for pronephric kidney organogenesis in *Xenopus laevis*. *The schematic representation shows a lateral view of the intermediate mesoderm (anterior to the left, dorsal up) depicting the development of the tubular and duct components of the pronephric kidney. For simplicity, the pronephric 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.*

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; Semba *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

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 morphogenesis. 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 mid-blastula stages (Nieuwkoop and Faber, 1994). Expression of *integrin–α6* 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-α6 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 chemoattractant 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 lumenal 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 ClC-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.

Acknowledgments

I thank Drs. M. Asashima, Y. Audigier, E. Bellefroid, C. Kintner, E. Jones, P. Krieg, T. Lallier, T. Mohun, R. Moon, M. Moos, P. Remy, S. Sato, K. Semba, D.-L. Shi, R. Stick, M. Taira and R. Vignali for providing plasmids; K. Tweedell for WMPA cells; students of the Swiss Federal Institute of Technology participating in the laboratory course "Experimentelle Biologie II" for performing whole-mount in situ hybridizations, and the members of the lab (S. Eid, H. Ghanbari, C. Halin, D. Saulnier, and A. Terrettaz) for providing stained embryos, performing photography, and critical comments on the manuscript. A.W.B. is a recipient of a Career Development Award of the Swiss National Science Foundation (START; No. 31-38807.93). Work in the author's laboratory is supported by grants of the Roche Research Foundation, Wolfermann-Nägeli-Stiftung Zürich, the Swiss Federal Institute of Technology, and the Swiss National Science Foundation (No. 31-50755.97).

References

ARTAVANIS-TSAKONAS, S., RAND, M.D. and LAKE, R.J. (1999). Notch signaling: cell fate control and signal integration in development. *Science 284:* 770-776.

ASASHIMA, M. (1994). Mesoderm induction during early amphibian development. *Dev. Growth Differ. 36:* 343-355.

BALINSKY, B.I. (1970). *An introduction to embryology*. Saunders, Philadelphia.

BELLAIRS, R., LEAR, R.P., YAMADA, K.M., RUTISHAUSER, U. and LASH, J.W. (1995). Posterior extension of the chick nephric (Wolffian) duct: the role of fibronectin and NCAM polysialic acid. *Dev. Dynamics 202:* 333-342.

BELLEFROID, E.J., KOBBE, A., GRUSS, P., PIELER, T., GURDON, J.B. and PAPALOPULU, N. (1998). *Xiro3* encodes a *Xenopus* homolog of the *Drosophila Iroquois* genes and functions in neural specification. *EMBO J. 17:* 191-203.

BERTEWISTLE, D., WALMSLEY, M.E., READ, E.M., PIZZEY, J.A. and PATIENT, R.K. (1996). GATA factors and the origins of adult and embryonic blood in *Xenopus*: responses to retinoic acid. *Mech. Dev. 57*: 199-214.

BLADT, F., RIETHMACHER, D., ISENMANN, S., AGUZZI, A. and BIRCHMEIER, C. (1995). Essential role for c-met receptor in the migration of myogenic precursor cells into the limb bud. *Nature 376:* 768-771.

BRENNAN, H.C., NIJJAR, S. and JONES, E.A. (1998). The specification of the pronephric tubules and duct in *Xenopus laevis*. *Mech. Dev. 75:* 127-137.

BURKITT, H.G., YOUNG, B. and HEATH, J.W. (1993). *Wheater's functional histology*. Churchill Livingstone, Edinburgh.

CALLAZO, A., BRONNER-FRASER, M. and FRASER, S.E. (1993). Vital dye labelling of *Xenopus laevis* trunk neural crest reveals multipotency and novel pathways of migration. *Development 118:* 363-376.

CARLSON, B.M. (1996). *Patten's foundation of embryology* - Sixth edition. McGraw-Hill, New York.

CARPENTER, K.L. and TURPEN, J.B. (1979). Experimental studies on hemopoiesis in the pronephros of *Rana pipiens*. *Differentiation 14*: 167-174.

CARROLL, T.J. and VIZE, P.D. (1996). Wilms' tumor suppressor gene is involved in the development of disparate kidney forms: evidence from expression in the *Xenopus* pronephros. *Dev. Dynamics 206:* 131-138.

CARROLL, T., WALLINGFORD, J., SEUFERT, D. and VIZE, P.D. (1999a). Molecular regulation of pronephric development. *Curr. Top. Dev. Biol. 44:* 67-100.

CARROLL, T.J., WALLINGFORD, J.B. and VIZE, P.D. (1999b). Dynamic patterns of gene expression in the developing pronephros of *Xenopus leavis*. *Dev. Genet. 24:* 199-207.

CHOI, K., KENNEDY, M., KAZAROV, A., PAPADIMITRIOU, J.C. and KELLER, G. (1998). A common precursor for hematopoietic and endothelial cells. *Development 125:* 725-732.

CLEAVER, O. and KRIEG, P.A. (1998). VEGF mediates angioblast migration during development of the dorsal aorta in *Xenopus*. *Development 125:* 3905-3914.

CLEAVER, O., TONISSEN, K.F., SAHA, M.S. and KRIEG, P.A. (1997). Neovascularization of the *Xenopus* embryo. *Dev. Dynamics 210:* 66-77.

DAVIES, J.A. and BARD, J.B.L. (1998). The development of the kidney. *Curr. Top. Dev. Biol. 39:* 245-301.

DAVIES, J.A. and BRÄNDLI, A.W. (1997). The Kidney Development Database. World Wide Web URL: http://www.ana.ed.ac.uk/anatomy/database/kidbase/kidhome.html.

DAWID, I.B. (1994). Intercellular signaling and gene regulation during early embryogenesis of *Xenopus laevis*. *J. Biol. Chem. 269:* 6259-6262.

DEMARTIS, A., MAFFEI, M., VIGNALI, R., BARSACCHI, G. and DE SIMONE, V. (1994). Cloning and developmental expression of LFB3/HNF1β transcription factor in *Xenopus laevis*. *Mech. Dev. 47:* 19-28.

DEVIC, E., PAQUEREAU, L., VERNIER, P., KNIBIEHLER, B. and AUDIGIER, Y. (1996). Expression of a new G protein-coupled X-msr is associated with an endothelial lineage in *Xenopus laevis*. *Mech. Dev. 59:* 129-140.

DOSCH, R., GAWANTKA, V., DELIUS, H., BLUMENSTOCK, C. and NIEHRS, C. (1997). BMP-4 acts as a morphogen in dorsoventral mesoderm patterning in *Xenopus*. *Development 124*: 2325-2334.

DRAWBRIDGE, J. and STEINBERG, M.S. (1996). Morphogenesis of the axolotl pronephric duct: a model system for the study of cell migration *in vivo*. *Int. J. Dev. Biol. 40:* 709-713.

DUCHATELLE, P., OHARA, A., LING, B.N., KEMENDY, A.E., KOKKO, K.E., MATSUMOTO, P.S. and EATON, D.C. (1992). Regulation of renal epithelial sodium channels. *Mol. Cell. Biochem. 114:* 27-34.

DUDLEY, A.T., LYONS, K.M. and ROBERTSON, E.J. (1995). A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. *Genes Dev. 9:* 2795-2807.

DZIERZAK, E. and MEDVINSKY, A. (1995). Mouse embryonic hematopoiesis. *Trends Genet. 11*: 359-366.

EKBLOM, P. (1992). Renal development. In *The kidney: physiology and pathophysiology* (Ed. D.W. Seldin and G. Giebisch). Raven Press, New York, pp. 475-501.

EKBLOM, P. and WELLER, A. (1991). Ontogeny of tubulointerstitial cells. *Kidney Int. 39:* 394-400.

FALES, D.E. (1935). Experiments on the development of the pronephros of *Amblystoma punctatum*. *J. Exp. Zool. 72:* 147-173.

FAVOR, J., SANDULACHE, R., NEUHÄUSLER-KLAUS, A., PRETSCH, W., CHATTERJEE, B., SENFT, E., WURST, W., BLANQUET, V., GRIMES, P., SPÖRLE, R. and SCHUGHART, K. (1996). The mouse *Pax2*^{1Neu} mutation is identical to a human *PAX2* mutation in a family with renal-coloboma syndrome and results in developmental defects of the brain, ear, eye, and kidney. *Proc. Natl. Acad. Sci. USA 93:* 13870-13875.

FELIX, W. and BÜHLER, A. (1906). Die Entwicklung des Harnapparates. In *Handbuch der vergleichenden und experimentellen Entwicklungslehre der Wirbeltiere* (Ed. O. Hertwig). Vol. 3. Gustav Fischer, Jena. pp. 81-442.

FOLKMAN, J. and D'AMORE, P.A. (1996). Blood vessel formation: what is its molecular basis? *Cell 87:* 1153-1155.

FOX, H. (1963). The amphibian pronephros. *Q. Rev. Biol. 38:* 1-25.

FRANK, G. (1988). Granulopoiesis in tadpoles of *Rana esculenta*. Survey of the organs involved. *J. Anat. 160:* 59-66.

GAUSH, C.R., HARD, W.L. and SMITH, T.F. (1966). Characterization of an established line of canine kidney cells (MDCK). *Proc. Soc. Exp. Biol. Med. 122:* 931-935.

GAWANTKA, V., POLLET, N., DELIUS, H., VINGRON, M., PFISTER, R., NITSCH, R., BLUMENSTOCK, C. and NIEHRS, C. (1998). Gene expression screening in *Xenopus* identifies molecular pathways, predicts gene function and provides a global view of embryonic patterning. *Mech. Dev. 77:* 95-141.

GEERING, K. (1997). Na, K-ATPase. *Curr. Opin. Nephrol. Hypertens. 6:* 434-439.

GERING, M., RODAWAY, A.R.F., GOTTGENS, B., PATIENT, R.K. and GREEN, A.R. (1998). The SCL gene specifies haemangioblast development from early mesoderm. *EMBO J. 17:* 4029-4045.

GOODRICH, E.S. (1930). *Studies on the structure and development of vertebrates.* MacMillan, London.

GRAFF, J.M. (1997). Embryonic patterning: to BMP or not to BMP, that is the question. *Cell 89:* 171-174.

GROBSTEIN, C. (1953). Inductive epithelio-mesenchymal interaction in cultured organ rudiments of the mouse. *Science 118:* 52-55.

HATINI, V., HUH, S.O., HERZLINGER, D., SOARES, V.C. and LAI, E. (1996). Essential role of stromal mesenchyme in kidney morphogenesis revealed by targeted disruption of winged helix transcription factor BF-2. *Genes Dev. 10:* 1467-1478.

HEASMANN, J. (1997). Patterning the *Xenopus* blastula. *Development 124:* 4179-4191.

HELLER, N. and BRÄNDLI, A.W. (1997). *Xenopus* Pax-2 displays multiple splice forms during embryogenesis and pronephric kidney development. *Mech. Dev. 69:* 83-104.

HELLER, N. and BRÄNDLI, A.W. (1999). *Xenopus* Pax-2/5/8 orthologues: novel insights into Pax gene evolution and identification of Pax-8 as the earliest marker for otic and pronephric cell lineages. *Dev. Genet. 24:* 208-219.

HENRIQUE, D., ADAM, J., MYAT, A., CHITNIS, A., LEWIS, J. and ISH-HOROWICZ, D. (1995). Expression of a *Delta* homologue in prospective neurons in the chick. *Nature 375:* 787-790.

HERZLINGER, D. (1995). Inductive interactions during kidney development. *Semin. Nephrol. 15:* 255-262.

HOLLEMANN, T., SCHUH, T., PIELER, T. and STICK, R. (1996). *Xenopus* Xsal-1, a vertebrate homolog of the region specific homeotic gene spalt of *Drosophila*. *Mech. Dev. 55:* 19-32.

HOLTFRETER, J. (1944). Experimental studies on the development of the pronephros. *Rev. Can. Biol. 3:* 220-250.

HSIEH, J.C., KODJABACHIAN, L., REBBERT, M.L., RATTNER, A., SMALLWOOD, P.M., SAMOS, C.H., NUSSE, R., DAWID, I.B. and NATHANS, J. (1999). A new secreted protein that binds to Wnt proteins and inhibits their activities. *Nature 398:* 431-436.

HSU, D., ECOMINEDIES, A., WANG, X., EIMON, P. and HARLAND, R.M. (1998). The *Xenopus* dorsalizing factor Gremlin identifies a new family of secreted proteins that antagonize BMP activities. *Mol. Cell 1:* 673-683.

HUBER, T.L. and ZON, L.I. (1998). Transcriptional regulation of blood formation during *Xenopus* development. *Semin. Immunol. 10:* 103-109.

JENA, N., MARTIN-SEISDEDOS, C., MCCUE, P. and CROCE, C.M. (1997). BMB7 null mutation in mice: developmental defects in skeleton, kidney, and eye. *Exp. Cell Res. 230:* 28-37.

JONES, S.D., HO, L., SMITH, J.C., YORDAN, C., STILES, C.D. and MERCOLA, M. (1993). The *Xenopus* platelet-derived growth factor a receptor: cDNA cloning and demonstration that mesoderm induction establishes the lineage-specific pattern of ligand and receptor gene expression. *Dev. Genet. 14:* 185-193.

KELLEY, C., YEE, K., HARLAND, R. and ZON, L.I. (1994). Ventral expression of GATA-1 and GATA-2 in the *Xenopus* embryo defines induction of hematopoietic mesoderm. *Dev. Biol. 165:* 193-205.

KESSLER, D.S. and MELTON, D.A. (1994). Vertebrate embryonic induction: mesodermal and neural patterning. *Science 266:* 596-604.

KISPERT, A., VAINIO, S., SHEN, L., ROWITCH, D.H. and MCMAHON, A.P. (1996). Proteoglycans are required for maintenance of *Wnt-11* expression in the ureter tips. *Development 122:* 3627-3637.

KOHLHASE, J., WISCHERMANN, A., REICHENBACH, H., FROSTER, U. and ENGEL, W. (1998). Mutations of the *SALL1* putative transcription factor gene cause Townes-Brocks syndrome. *Nat. Genet. 18:* 81-83.

KOSTER, M., DILLINGER, K. and KNÖCHEL, W. (1998). Expression pattern of the winged helix factor XFD-11 during *Xenopus* embryogenesis. *Mech. Dev. 76:* 169-173.

KÖSTER, R., STICK, R., LOOSLI, F. and WITTBRODT, J. (1997). Medaka *spalt* acts as a target gene of hedgehog signaling. *Development 124:* 3147-3156.

KREIDBERG, J.A., SARIOLA, H., LORING, J.M., MAEDA, M., PELLETIER, J., HOUSMAN, D. and JAENISCH, R. (1993). WT-1 is required for early kidney development. *Cell 74:* 679-691.

KROTOSKI, D.M., FRASER, S.E. and BRONNER-FRASER, M. (1988). Mapping of neural crest pathways in *Xenopus laevis* using inter- and intra-specific cell markers. *Dev. Biol. 127:* 119-132.

LALLIER, T.E., WHITTAKER, C.A. and DESIMONE, D.W. (1996). Integrin a6 expression is required for early nervous system development in *Xenopus laevis*. *Development 122:* 2539-2554.

LECHNER, M.S. and DRESSLER, G.R. (1997). The molecular basis of embryonic kidney development. *Mech. Dev. 62:* 105-120.

LEVÉEN, P., PEKNY, M., GEBRE-MEDHIN, S., SWOLIN, B., LARSSON, E. and BETSHOLTZ, C. (1994). Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. *Genes Dev. 8*: 1875-1887.

LINDENTHAL, S., SCHMIEDER, S., EHRENFELD, J. and WILLS, N.K. (1997). Cloning and functional expression of a ClC Cl^- channel from the renal cell line A6. *Am. J. Physiol. 273:* C1176-C1185.

LUO, G., HOFMANN, C., BRONCKERS, A.L.J.J., SOHOCKI, M., BRADLEY, A. and KARSENTY, G. (1995). BMP-7 is an inducer of nephrogenesis, and is also required for eye development and skeletal patterning. *Genes Dev. 9*: 2808-2820.

LYNCH, K. and FRASER, S.E. (1990). Cell migration in the formation of the pronephric duct in *Xenopus laevis*. *Dev. Biol. 142*: 283-292.

MAJUMDAR, A. and DRUMMOND, I.A. (1999). Podocyte differentiation in the absence of endothelial cells as revealed in the zebrafish avascular mutant, *cloche*. *Dev. Genet. 24*: 220-229.

McGREW, L.L., OTTE, A.P. and MOON, R.T. (1992). Analysis of *Xwnt-4* in embryos of *Xenopus laevis*: A *Wnt* family member expressed in the brain and floor plate. *Development 115:* 463-473.

McKINNELL, R.G. (1994). Reduced oncogenic potentential associated with differentiation of the Lucké renal adenocarcinoma. *In Vivo 8:* 65-70.

MEAD, P.E., KELLEY, C.M., HAHN, P.S., PIEDAD, O. and ZON, L.I. (1998). SCL specifies hematopoietic mesoderm in *Xenopus* embryos. *Development 125:* 2611-2620.

MEYER, D., DURLIAT, M., SENAN, F., WOLFF, M., ANDRE, M., HOURDRY, J. and REMY, P. (1997). Ets-1 and Ets-2 proto-oncogenes exhibit differential and restricted expression patterns during *Xenopus laevis* oogenesis and embryogenesis. *Int. J. Dev. Biol. 41:* 607-620.

MILLARD, N. (1949). The development of the venous system of *Xenopus laevis*. *Trans. Roy. Soc. S. Africa 32:* 55-99.

MONTESANO, R., MATSUMOTO, K., NAKAMURA, T. and ORCI, L. (1991a). Identification of a fibroblast-derived epithelial morphogen as hepatocyte growth factor. *Cell 67:* 901-908.

MONTESANO, R., SCHALLER, G. and ORCI, L. (1991b). Induction of epithelial tubular morphogenesis *in vitro* by fibroblast-derived soluble factors. *Cell 66:* 697-711.

MOORE, A.W., MCINNES, L., KREIDBERG, J., HASTIE, N.D. and SCHEDL, A. (1999). YAC complementation shows a requirement for *Wt1* in the development of epicardium, adrenal gland and throughout nephrogenesis. *Development 126:* 1845-1857.

MORIYA, N., UCHIYAMA, H. and ASASHIMA, M. (1993). Induction of pronephric tubules by activin and retinoic acid in presumptive ectoderm of *Xenopus laevis*. *Dev. Growth Differ. 35:* 123-128.

MÜLLER, U. and BRÄNDLI, A.W. (1999). Cell adhesion molecules and extracellular matrix constituents in kidney development and disease. *J. Cell Science*.

MURRE, C., BAIN, G., VAN DIJK, M.A., ENGEL, I., FURNARI, B.A., MASSARI, M.E., MATHEWS, J.R., QUONG, M.W., RIVERA, R.R. and STUIVER, M.H. (1994). Structure and function of helix-loop-helix proteins. *Biochem. Biophys. Acta 1218:* 129-135.

NIEUWKOOP, P.D. and FABER, J. (1994). *Normal table of Xenopus laevis (Daudin): a systematical and chronological survey of the development from the fertilized egg till the end of metamorphosis*. Garland Publishing, Inc., New York & London.

NORTON, J.D., DEED, R.W., CRAGGS, G. and SABLITZKY, F. (1998). Id helix-loop-helix proteins in growth and differentiation. *Trends Cell. Biol. 8:* 58-65.

OBARA-ISHIHARA, T., KUHLMANN, J., NISWANDER, L. and HERZLINGER, D. (1999). The surface ectoderm is essential for nephric duct formation in intermediate mesoderm. *Development 126:* 1103-1108.

PANNESE, M., LUPO, G., KABLAR, B., BONCINELLI, E., BARSACCHI, G. and VIGNALI, R. (1997). The *Xenopus Emx* genes identify presumptive dorsal telencephalon and are induced by head organizer signals. *Mech. Dev. 73:* 73-83.

PARDANAUD, L., LUTON, D., PRIGENT, M., BOURCHEIX, L.M., CATALA, M. and DIETERLEN-LIÈVRE, F. (1996). Two distinct endothelial lineages in ontogeny, one of them related to hemopoiesis. *Development 122:* 1363-1371.

PASTEELS, J. (1942). New observations concerning the maps of presumptive areas in amphibian gastrula (*Amblystoma* and *Discoglossus*). *J. Exp. Zool. 89:* 255-281.

PRICE, M.P., ISHIHARA, H., SHEPPARD, D.N. and WELSH, M.J. (1996). Function of *Xenopus* cystic fibrosis transmembrane conductance regulator (CFTR) Cl channels and use of human-*Xenopus* chimeras to investigate the pore properties of CFTR. *J. Biol. Chem. 271*: 25184-25191.

PUOTI, A., MAY, A., CANNESSA, C.M., HORISBERGER, J.-D., SCHILD, L. and ROSSIER, B.C. (1995). The highly selective low-conductance epithelial Na channel of *Xenopus laevis* A6 kidney cells. *Am. J. Physiol. 38:* C188-197.

PUOTI, A., MAY, A., ROSSIER, B.C. and HORRISBERGER, J.-D. (1997). Novel isoforms of the β and γ subunits of the *Xenopus* epithelial Na channel provide information about amiloride binding site and extracellular sodium sensing. *Proc. Natl. Acad. Sci. USA 94:* 5949-5954.

RAFFERTY, K.A. (1969). Mass culture of amphibian cells: methods and observations concerning the stability of cell type. In *Biology of amphibian tumors* (Ed. M. Mizell). Springer-Verlag, Berlin. pp. 52-81.

RISAU, W. (1997). Mechanisms of angiogenesis. *Nature 386:* 671-674.

RISAU, W. and FLAMME, I. (1995). Vasculogenesis. *Annu. Rev. Cell Dev. Biol. 11:* 73-91.

RUVKUN, G. and HOBERT, O. (1998). The taxonomy of developmental control in *Caenorhabditis elegans*. *Science 282:* 2033-2041.

SARIOLA, H. and SAINIO, K. (1997). The tip-top branching ureter. *Curr. Opin. Cell Biol. 9*: 877-884.

SAXÉN, L. (1987). *Organogenesis of the kidney*. Cambridge University Press, Cambridge, UK.

SCHMIDT, C., BLADT, F., GOEDECKE, S., BRINKMANN, V., ZSCHIESCHE, W., SHARPE, M., GHERARDI, E. and BIRCHMEYER, C. (1995). Scatter factor/ hepatocyte growth factor is essential for liver development. *Nature 373:* 699-702.

SEMBA, K., SAITO-UENO, R., TAKAYAMA, G. and KONDO, M. (1996). cDNA cloning and its pronephros-specific expression of the Wilm's tumor suppressor gene, WT1, from *Xenopus leavis. Gene 175:* 167-172.

SHAWLOT, W. and BEHRINGER, R.R. (1995). Requirement for Lim1 in head-organizer function. *Nature 374:* 425-430.

SHI, D.L., GOISSET, C. and BOUCAUT, J.C. (1998). Expression of Xfz3, a *Xenopus* frizzled family member, is restricted to the early nervous system. *Mech. Dev. 70:* 35-47.

SIDOW, A. (1996). Gen(om)e duplications in the evolution if early vertebrates. *Curr. Opin. Genet. Dev. 6:* 715-722.

SLACK, J.M.W. (1994). Inducing factors in *Xenopus* early embryos. *Curr. Biol. 4:* 116-126.

SORIANO, P. (1994). Abnormal kidney development and hematological disorders in PDGF b-receptor mutant mice. *Genes Dev. 8:* 1888-1896.

STARK, K., VAINO, S., VASSILEVA, G. and MCMAHON, A.P. (1994). Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4. *Nature 372:* 679-683.

STENNARD, F., RYAN, K. and GURDON, J.B. (1997). Markers of vertebrate mesoderm induction. *Curr. Opin. Genet. Dev. 7:* 620-627.

STEVENSON, B.R. and KEON, B.H. (1998). The tight junction: morphology to molecules. *Annu. Rev. Cell Dev. Biol. 14:* 89-109.

TAHARA, T., OGAWA, K. and TANIGUCHI, K. (1993). Ontogeny of the pronephros and mesonephros in the South African clawed frog, *Xenopus laevis* Daudin, with special reference to the appearance and movements of the renin-immunopositive cells. *Exp. Anim. 42:* 601-610.

TAIRA, M., OTANI, H., JAMRICH, M. and DAWID, I.B. (1994). Expression of the LIM class homeobox gene *XLIM-1* in pronephros and CNS cell lineages of *Xenopus* embryos is affected by retinoic acid and exogastrulation. *Development 120:* 1525-1536.

TORRES, M., GÓMEZ-PARDO, E., DRESSLER, G.R. and GRUSS, P. (1995). Pax-2 controls multiple steps of urogenital development. *Development 121*: 4057-4065.

TSAI, F.Y., KELLER, G., KUO, F.C., WEISS, M.J., CHEN, J.-Z., ROSENBLATT, M., ALT, F. and ORKIN, S.H. (1994). An early haematopoietic defect in mice lacking the transcription factor GATA-2. *Nature 371*: 221-226.

TUCKER, S.J., TANNAHILL, D. and HIGGINS, C.F. (1992). Identification and developmental expression of *Xenopus laevis* cystic fibrosis transmembrane conductance regulator gene. *Hum. Mol. Genet. 2:* 77-82.

TURPEN, J. and KNUDSON, C. (1982). Ontogeny of hemopoetic cells in *Rana pipiens*: precursor cell migration during embryogenesis. *Dev. Biol. 104:* 138-150.

TURPEN, J.B., KELLEY, C.M., MEAD, P.E. and ZON, L.I. (1997). Bipotential primitive-definitive hematopoietic progenitors in the vertebrate embryo. *Immunity 7:* 325-334.

TWEEDELL, K.S. and WONG, W.Y. (1974). Frog kidney tumors induced by herpes virus cultured in pronephric cells. *J. Natl. Cancer Inst. 52:* 621-624.

UEHARA, Y., MINOW, O., MORI, C., SHIOT, K., JUNO, J. and KITAMURA, N. (1995). Placental defect and embryonic lethality in mice lacking hepatocyte growth factor/ scatter factor. *Nature 373:* 702-705.

UOCHI, T. and ASASHIMA, M. (1996). Sequential gene expression during pronephric tubule formation *in vitro* in *Xenopus* ectoderm. *Dev. Growth Differ. 38:* 625-634.

UOCHI, T., TAKAHASHI, S., NINOMIYA, H., FUKUI, A. and ASASHIMA, M. (1997). The Na^+, K^+-ATPase α subunit requires gastrulation in the *Xenopus* embryo. *Dev. Growth Differ. 39:* 571-580.

VAINIO, S. and MÜLLER, U. (1997). Inductive tissue interactions, cell signalling, and the control of kidney organogenesis. *Cell 90:* 975-978.

VIZE, P.D., JONES, E.A. and PFISTER, R. (1995). Development of the *Xenopus* pronephric system. *Dev. Biol. 171:* 531-540.

VIZE, P.D., SEUFERT, D.W., CARROLL, T.J. and WALLINGFORD, J.B. (1997). Model systems for the study of kidney development: use of the pronephros in the analysis of organ induction and patterning. *Dev. Biol. 188:* 189-204.

WALLINGFORD, J.B., CARROLL, T.J. and VIZE, P.D. (1998). Precocious expression of the Wilm's tumor gene xWT1 inhibits embryonic kidney development in *Xenopus leavis. Dev. Biol 202:* 103-112.

WALMSLEY, M.E., GUILLE, M.J., BERTWISTLE, D., SMITH, J.C., PIZZEY, J.A. and PATIENT, R.K. (1994). Negative control of *Xenopus* GATA-2 by activin and noggin with the eventual expression in precursors of the ventral blood islands. *Development 120:* 2519-2529.

WANG, S., KRINKS, M., KLEINWAKS, L. and MOOS, J., M. (1997). A novel *Xenopus* homologue of bone morphogenetic protein-7 (BMP-7). *Genes Funct. 1:* 259-271.

WILSON, R. and MOHUN, T. (1995). XIdx, a dominant negative regulator of bHLH function in early *Xenopus* embryos. *Mech. Dev. 49:* 211-222.

WITTA, S.A., AGARWAL, V.R. and SATO, S.M. (1995). *XIPOU 2*, a noggin-inducible gene, has direct neuralizing activity. *Development 121:* 721-730.

WONG, W.Y. and TWEEDELL, K.S. (1974). Two viruses from the Lucké tumor isolated in a frog pronephric cell line. *Proc. Soc. Exp. Biol. Med. 145:* 1201-1206.

YEAMAN, C., GRINDSTAFF, K.K. and NELSON, W.J. (1999). New perspectives on mechanisms involved in generating epithelial cell polarity. *Physiol. Rev. 79*: 73-98.

ZACKSON, S.L. and STEINBERG, M.S. (1989). Axolotl pronephric duct cell migration is sensitive to phosphatidylinositol-specific phospholipase C. *Development 105:* 1-7.

ZON, L.I. (1995). Developmental biology of hematopoiesis. *Blood 86:* 2876-2891.