Metanephric Rat-Mouse Chimeras to Study Cell Lineage of the Nephron

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ABSTRACT The nephron is derived from the ureteric bud and metanephric mesenchyme and develops into a complex epithelial structure with a wide variety of phenotypes along its length. This segmental variation in expression of molecules provides an approach to understand the lineage of unique segments. The present study evaluated the expression of four relatively well-localized molecules — renin, Tamm-Horsfall protein (THP), oxytocin receptor (OTR), and the vasopressin type 2 receptor (V2R) — in cultured mouse-rat chimeric metanephric kidneys using reverse transcription-polymerase chain reaction (RT-PCR). Chimeric kidneys were formed by 1) separating the ureteric bud (U) from the metanephric mesenchyme (M) of mouse (m) at E11 and rat (r) at E13 days of gestation and 2) recombining the ureteric bud of one species with the metanephric mesenchyme of the other species (i.e., UrMm and UmMr), followed by filter culture until differentiated. Species-specific restriction enzymes for all four genes were chosen to digest the PCR product from either rat or mouse. RT-PCR was performed for each mRNA species and the products digested. The V2R product from the UrMm chimera was cleaved by a restriction enzyme known to digest only rat product, suggesting the PCR product was produced predominantly by cells derived from the ureteric bud. The renin, OTR, and THP products from both chimeras were cleaved equally well by species-specific restriction enzymes, suggesting the products were made by cells originating from both the ureteric bud and the metanephric mesenchyme. These studies demonstrate that the cultured chimeric metanephric model is useful to study segment lineage. The results suggest that the lineage of at least some portions of the nephron is heterogeneous. The proximal tubule, loop of Henle, and at least some parts of the distal tubule derive from the metanephric mesenchyme, while the collecting ducts originate from the ureteric bud. It is unclear exactly where the transition from mesenchyme- to ureteric bud-derived structures occurs, or if there is a distinct transition. In 1905, Huber concluded that the ureteric bud produced only the collecting duct and that all other portions of the nephron were derived from the mesenchyme. Later studies supported this view [Oliver, 1968; Potter, 1972]. Howie et al. [1993] suggested a more proximal transition zone, such that the transition would occur between the end of the distal tubule and the beginning of the connecting tubule. Recent studies [Herzlinger et al., 1992; Qiao et al., 1995], however, suggest a more plastic system allowing intermixing of mesenchyme- and ureteric bud-derived cells throughout the nephron. Many different approaches may be necessary to fully understand the contributions of the mesenchyme and ureteric bud to the various portions of the nephron. One approach, as described here, is to exploit the phenotypic variations in the different segments of the nephron by using molecules that have a relatively well-localized pattern of expression.

In the mature and developing kidney, renin, Tamm-Horsfall protein (THP), the oxytocin receptor (OTR), and the vasopressin type 2 receptor (V2R) have relatively restricted expression patterns which are well defined. Renin is very specifically expressed in the periglomerular arterioles and in the proximal tubule and the metanephric mesenchyme. These studies demonstrate that the cultured chimeric metanephric model is useful to study segment lineage. The results suggest that the lineage of at least certain portions of the nephron is heterogeneous. Dev. Genet. 24:230–240, 1999. ©1999 W. Iley-Liss, Inc.

KEY WORDS: kidney; development; vasopressin receptor; Tamm-Horsfall protein; renin; oxytocin receptor

INTRODUCTION The epithelial portions of the kidney are derived from two embryonic cell types, the metanephric mesenchyme and the ureteric bud. Based on early microdissection studies, it is generally accepted that the glomerulus, the proximal tubule, loop of Henle, and at least some parts of the distal tubule derive from the metanephric mesenchyme, while the collecting ducts originate from the ureteric bud. It is unclear exactly where the transition from mesenchyme- to ureteric bud-derived structures occurs, or if there is a distinct transition. In 1905, Huber concluded that the ureteric bud produced only the collecting duct and that all other portions of the nephron were derived from the mesenchyme. Later studies supported this view [Oliver, 1968; Potter, 1972]. Howie et al. [1993] suggested a more proximal transition zone, such that the transition would occur between the end of the distal tubule and the beginning of the connecting tubule. Recent studies [Herzlinger et al., 1992; Qiao et al., 1995], however, suggest a more plastic system allowing intermixing of mesenchyme- and ureteric bud-derived cells throughout the nephron. Many different approaches may be necessary to fully understand the contributions of the mesenchyme and ureteric bud to the various portions of the nephron. One approach, as described here, is to exploit the phenotypic variations in the different segments of the nephron by using molecules that have a relatively well-localized pattern of expression.

In the mature and developing kidney, renin, Tamm-Horsfall protein (THP), the oxytocin receptor (OTR), and the vasopressin type 2 receptor (V2R) have relatively restricted expression patterns which are well defined. Renin is very specifically expressed in the periglomerular arterioles and in the proximal tubule in the adult [Taugner et al., 1982]. In the embryonic kidney, renin is found in the arcuate and interlobar arteries, as well as in the afferent arterioles [Reddi et al., 1998]. The V2R is expressed in the collecting duct and thick ascending limb (TAL) in mature kidneys [Ostrowski et al., 1992] and is restricted to the collect-
ing duct structures in the developing metanephrons [Ostrowski et al., 1993]. In the adult, THP is expressed in the TAL of Henle's loop and in some cells of the early distal convoluted tubule [Sikri et al., 1981; Howie and Johnson, 1992]. The same expression pattern is found in the embryonic kidney when these segments of the nephron begin developing [Howie et al., 1993]. The OTR is restricted in expression in the mature kidney to the macula densa and a few cells of the distal convoluted tubule [Ostrowski et al., 1995]. This pattern of expression is the same in the embryonic kidney with additional expression in the developing TAL [Schmidt et al., 1990; Ostrowski et al., 1995].

With chimeric kidneys produced from the ureteric bud of one species and mesenchyme of another species, using reverse transcription-polymerase chain reaction (RT-PCR) and species-specific restriction enzymes, we were able to determine the species of origin and the embryonic progenitor of the cells expressing renin, OTR, THP, and V2R mRNA. The results indicate that the lineage of the cells expressing these molecules is not confined to a single embryonic cell type.

**MATERIALS AND METHODS**

**Animals and Dissection**

Timed-pregnant mice and rats were obtained from Harlan Sprague Dawley (Indianapolis, IN) and sacrificed by cervical dislocation at 11.5 days of gestation for the mouse and 13.5 days for the rat (day zero of gestation designated as day of vaginal plug). A midline abdominal incision was made to expose the uterine horns which were removed aseptically and placed in cold culture medium (Dulbecco's modified Eagle's medium [DMEM] supplemented with 10% fetal calf serum, 2 mM glutamine, 1 M dexamethasone, and 50 g/ml each penicillin and streptomycin). Embryos were removed from the placental membranes and placed in cold phosphate buffered saline (PBS; 8 mM NaH₂PO₄·H₂O, 140 mM NaCl, 3 mM KCl, 1.5 mM KH₂PO₄, pH 7.43) for dissection of the metanephroi. Embryonic kidneys were cultured intact, or in chimeric form with the ureteric bud from one species and the mesenchyme from the other species, using a modification of the transfilter system developed by Grobstein [1956]. Kidneys were placed on filters (polycarbonate, Costar) sitting atop a metal screen with a central hole, in six-well culture plates. The volume of the culture medium was adjusted to wet the filter without submerging the tissue. The kidneys were incubated at 37°C with 5% CO₂.

To develop cultured chimeric metanephric kidneys, the ureteric bud was removed from the metanephric mesenchyme by a 5-minute incubation of the intact kidney in 1 mM EDTA in PBS containing 10 U DNase/ml, followed by manual separation. Both portions were then incubated for 5 minutes in culture medium. The ureteric bud from one species was transferred to the culture apparatus, then the metanephric mesenchyme from the other species was transferred onto the filter and manually overlaid onto the ureteric bud. Chimeras were incubated under the same conditions described above. However, they were generally kept in culture 2–3 days longer than intact kidneys as they lagged slightly in branching of the ureteric bud and differentiation of the mesenchyme. Chimeras produced by combining the ureteric bud (U) from the rat (r) with the metanephric mesenchyme (M) from the mouse (m) are designated UrMm, while the opposite cross is designated UmMr.

**Confocal Microscopy**

To evaluate the progression of development of the chimeric metanephric kidneys in culture, they were compared to intact metanephric kidneys by confocal microscopy. Briefly, while attached to the filters, the kidneys were washed in PBS to remove the culture medium. They were fixed in 2% paraformaldehyde in PBS, pH 7.4, for 10 minutes at room temperature and stored in 0.5% paraformaldehyde in PBS at 4°C until use. The filters were washed twice in PBS and the kidneys permeabilized with 0.3% Triton X-100 in PBS for 5 minutes. The kidneys were incubated in propidium iodide (0.1 mg/ml PBS) for 5 minutes at room temperature. The kidneys were then removed from the filters and mounted in Vectorshield (Vector Labs) between two coverslips. Images were obtained on a Bio-Rad (Hercules, CA) MRC 600 laser scanning confocal microscope with a YHS filter set containing a 568 DF 10 excitor filter, a 585 DRLP dichroic reflector, and a 585 EFLP emission filter. Images were processed with Adobe Photoshop software.

**Determination of mRNA Expression by RT-PCR**

To determine mRNA expression in the cultured metanephric kidneys, intact or chimeric kidneys were cultured as described above for 6 to 8 days, removed from the filter, snap-frozen in guanidine isothiocyanate solution, and stored at -80°C until use. Total RNA was then isolated using a discontinuous gradient of cesium chloride, with Escherichia coli ribosomal RNA as carrier. cDNA was synthesized by incubating RNA at 42°C for 1 hour with H-MMLV reverse transcriptase (Superscript; BRL, Gaithersburg, MD), oligo dT (Pharmacia, Piscataway, NJ) as primer, RNAsin (Promega, Madison, WI), dithiothreitol, dNTPs (Pharmacia), and bovine serum albumin (BSA; Boehringer Mannheim, Indianapolis, IN). The cDNA was precipitated with linear acrylamide and resuspended in Tris-EDTA buffer.

For amplification, PCR reactions were performed in a total volume of 50 µl in the presence of 0.1 mM dNTP, 10 mM dithiothreitol, 50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris (pH 8.3), and 0.001% gelatin, with 0.5 pmol of each primer, 1.25 U Taq DNA Polymerase (Perkin-Elmer Cetus, Norwalk, CT), 1.5 µCi [3P]dCTP (Amersham, Arlington Heights, IL), and 1–6 µl of tissue cDNA.
Mineral oil was layered on top of each sample to prevent evaporation. After an initial denaturation at 94°C for 3.5 minutes, PCR amplification was performed for 30 cycles at 94°C (denaturation) for 1 minute, 54°C (annealing) for 1.5 minutes, and 72°C (extension) for 1 minute, followed by incubation at 72°C for 8 minutes. Primers were chosen from published sequences in an area of homology between the rat and mouse sequence to allow amplification of both species with the same primer pair. Sequences of all primers used and the expected sizes of amplification products are summarized in Table 1 [Yu et al., 1994; Lolait et al., 1992; Burnham et al., 1987; Rozen et al., 1995; Gunning et al., 1983]. 

Restriction Enzyme Digestion of PCR Products

PCR products were precipitated as described above and resuspended in dH2O. Five microliters of product was incubated at 37°C in a total volume of 10 µl containing restriction enzyme, 10X restriction buffer, and BSA. Restriction enzymes that could produce digestion of either rat or mouse product, but not both species, were chosen based on computer-generated restriction maps of the PCR product sequences. Restriction digestion was initially performed on the PCR product from mouse and rat intact cultured metanephric kidneys to confirm digestion of only one of the products. Enzymes which were proven to differentiate between the species were then used to digest PCR product from chimeric kidneys, to determine the species of origin of the product. Restriction fragments of chimeric product were analyzed by densitometry to determine the percentage of product in the fragments relative to the total product.

RESULTS

Development of Chimeric Metanephric Kidneys

Figure 1 shows a representative chimeric metanephric kidney after 7 days in culture. While the chimeric kidneys are somewhat disordered and lack the stereotyped, repetitive development seen in intact explants, they do develop well-defined comma- and S-shaped bodies and there is extensive branching of the ureteric bud. Metanephric mesenchyme prepared in the same manner, but cultured without a ureteric bud, underwent apoptosis within 2–3 days of explantation (results not shown). Likewise, ureteric bud dissected free of mesenchyme, identical to that obtained for chimeric cultures, failed to branch or produce tubular structures or nascent glomeruli when cultured alone (results not shown). As shown previously [Gruenwald, 1943; Sariola...
et al., 1989], isolated metanephric mesenchyme, when cultured with spinal cord tissue, also developed nascent glomerular structures.

**Restriction Digestion of Renin PCR Product**

The restriction enzymes EcoRI and BstXI were chosen to differentiate between mouse and rat renin PCR products based on the restriction maps for each species. The results of digestion of renin PCR product from either mouse or rat intact cultured metanephric kidneys are shown in Figure 2.

As expected, EcoRI cut the rat product (600 bp uncut) into two fragments of 100 and 500 bp, but did not digest the mouse product. BstXI cut the mouse renin product into 85 and 515 bp fragments but failed to cut the rat renin product.

**Origin of Renin PCR Product From Chimeric Metanephric Kidneys**

Renin PCR product from chimeric kidneys was subjected to restriction digestion with EcoRI and BstXI. The PCR product from both chimeras (Fig. 3) was digested equally well by both enzymes. By densitometry, 53.9 ± 3.8% of the renin product from UₐMₐ chimeras (n = 8) was found in the restriction fragments when EcoRI was used and 54.1 ± 5.1% was digested when BstXI was used. Similar results were found with the UₐMₐ chimeras (n = 4). When EcoRI was used to digest the UₐMₐ chimeras, 52.7 ± 6.5% of the product was found in the fragments, while BstXI digestion of the renin product from UₐMₐ chimeras resulted in 55.9 ± 1.8% of the product in fragments. These results indicate that the renin mRNA present was produced by
cells originating from both species. Therefore, these cells derived from both the metanephric mesenchyme and ureteric bud.

Restriction Digestion of OTR PCR Product

The restriction enzymes BanI and NciI were chosen to differentiate between mouse and rat OTR PCR products based on the restriction maps for each species (Fig. 4A). The results of digestion of OTR PCR product from either mouse or rat intact cultured metanephric kidneys are shown in Figure 4.

As expected, BanI cut the mouse product (288 bp uncut) into two fragments of 129 and 159 bp (Fig. 4B), but did not digest the rat product. NciI cut the rat OTR product into 111 and 177 bp fragments but failed to cut the mouse OTR product.

Origin of OTR PCR Product From Chimeric Metanephric Kidneys

OTR PCR product from chimeric cultured kidneys was subjected to restriction digestion with BanI and NciI. The PCR product from both chimeras (Fig. 5A) was digested equally well by both enzymes. By densitometry, 60.6 ± 3.6% of the OTR product from UrMm

Fig. 3. Restriction digestion of chimeric metanephric kidney renin PCR product. A: Autoradiogram of 32P-labeled renin PCR product from representative UrM_m and UmM_r chimeras after digestion with EcoRI and BstXI. Undigested product is 600 bp. Approximately 55% of the renin product from either type of chimera was found in the fragments when either BstXI (B) or EcoRI (E) was used. B: In either chimeric metanephric kidney type, both mouse and rat contributed to the renin product, suggesting the cellular derivation was both metanephric mesenchyme and ureteric bud.

Fig. 4. Restriction digestion of OTR PCR product from intact mouse and rat cultured metanephric kidneys. A: Expected digestion pattern of mouse and rat OTR product by BanI and NciI based on restriction maps for each species. Undigested product size is 288 bp. BanI would be expected to cleave only mouse OTR PCR product into 129 and 159 bp fragments, while NciI should cut only the rat product (111 and 177 bp fragments). B: OTR PCR product from intact mouse and rat cultured metanephric kidneys digested with BanI and NciI. The mouse product is cut by BanI and the rat product is cut by NciI as expected from the restriction maps.
chimeras (n = 13) was found in the restriction fragments when BanI was used and 55.9 ± 3.7% was digested when NciI was used. Similar results were found with the U rMm chimeras (n = 16). When BanI was used to digest the U rMm chimeras, 62.9 ± 1.9% of the product was found in the fragments, whereas NciI digestion of the OTR product from U rMm chimeras resulted in 58.6 ± 2.3% of the product in the fragments.

Sequence of Mouse V2R Receptor PCR Product

The mouse homologue of the PCR product for V2R was cloned and the sequence is shown in Figure 6 compared to the published sequence for rat [Lodait et al., 1992]. The mouse sequence is 97% identical to the rat at the nucleotide level in this area of the molecule. This sequence was used to generate a restriction enzyme map for the mouse V2R PCR product to identify species-specific restriction enzymes.

Restriction Digestion of the V2R PCR Product

The restriction enzyme NheI was chosen to differentiate between mouse and rat V2R PCR products based on the restriction maps for each species (Fig. 7A). Results of NheI digestion of the V2R product from intact mouse and rat cultured metanephric kidneys are shown in Figure 7B. NheI cleaved the rat V2R product into 166 and 150 bp fragments, but as expected, did not cut the mouse product (undigested product size: 316 bp).

Origin of V2R PCR Product From Chimeric Metanephric Kidneys

V2R PCR product from chimeric cultured kidneys was subjected to restriction digestion with NheI. The PCR product from chimeras of the U rMm type (Fig. 8, left panel; n = 7) was digested by NheI to produce fragments of the expected size. The band at approximately 600 bp represents a doublet of the 316 bp V2R PCR product. By densitometry, 77.3 ± 7.6% of the V2R product from U rMm chimeras was found in the restriction fragments. These results are consistent with product derived predominantly from the rat portion of the U rMm chimera, i.e., the ureteric bud. Alternatively, 33.4 ± 7.8% of the V2R product from U mMr chimeras (Fig. 8, right panel; n = 9) was found in the restriction fragments, leaving nearly 70% uncult, consistent with the PCR product derived predominantly from the mouse portion in this chimera, i.e., the ureteric bud. Differential restriction enzyme digestion of these two chimeras by NheI consistently demonstrated that the V2R mRNA present in these chimeras predominantly derived from the ureteric bud, rather than from the metanephric mesenchyme.
Sequence of Mouse THP PCR Product

The mouse homologue of the PCR product for THP was cloned and the sequence is shown in Figure 9 compared to the published sequence for rat [Yu et al., 1994]. The mouse sequence is 92% identical to the rat at the nucleotide level in this area of the molecule. A restriction enzyme map of this mouse sequence for the THP PCR product was generated to identify species-specific enzymes.

Restriction Digestion of THP PCR Product

The restriction enzyme BanII was chosen to differentiate between mouse and rat THP PCR product (Fig. 10A). Results of BanII digestion of the THP product from intact mouse and rat cultured metanephric kidneys are shown in Figure 10B. BanII cleaved the THP product from the mouse into 270 and 130 bp fragments, but as expected, did not cut rat product (400 bp).
Origin of THP PCR Product From Chimeric Metanephric Kidneys

THP PCR product from cultured chimeric kidneys was subjected to restriction digestion with BanII. THP product from chimeras of both types (Fig. 11A) was digested by BanII to a similar degree. By densitometry, 61.6 ± 3.4% of the THP product from UrMm chimeras (n = 16) was found in the restriction fragments and 70.9 ± 2.2% of the THP product from UmMr chimeras (n = 14) was cut by BanII. Since BanII is capable of cutting only the mouse THP product, and just over one half of the PCR product is found in the restriction fragments in both chimeras, these results are consistent with THP product derived from both the metanephric mesenchyme and the ureteric bud.

DISCUSSION

The phenotypic variation of the nephron is striking; there are at least 16 different types of epithelium from the glomerulus to the collecting duct. The distinctions are evident at the ultrastructural level as well as at the functional level, with wide-ranging differences in transport capabilities and hormone responsiveness. All of these diverse cellular phenotypes evolve from two simple embryologic cell types, undifferentiated metanephric mesenchyme, and an epithelial outgrowth of the Wolffian duct, the ureteric bud. Classically, the nephron has been viewed as two distinct groups of cells, i.e., those that derive from undifferentiated mesenchyme and those that derive from ureteric bud cells. Controversy remains regarding the point in the nephron where mesenchyme-derived cells end and ureteric bud-derived cells begin. Indeed, there is recent evidence to suggest that this “segmentation” is somewhat artificial and cells derived from either embryonic precursor may be located at any point along the nephron [Herzlinger et al., 1992; Qiao et al., 1995]. Classically, it has been accepted that the collecting system per se is derived from the ureteric bud, while the remaining nephron is derived from differentiation of the metanephric mesenchyme into epithelial cells. With minor differences in the exact location of the transition from mesenchyme- to ureteric bud-derived epithelium, Ol-
iver [1968], Potter [1972], and Howie et al. [1993] have all placed this region within the distal nephron, somewhere between the beginning of the distal convoluted tubule (DCT) and the beginning of the collecting duct. By using LacZ-tagged retroviruses, others have provided support for the hypothesis that a distinct transition point in fact does not exist and there is a mixture of mesenchyme- and ureteric bud-derived cells throughout the nephron [Herzlinger et al., 1992; Qiao et al., 1995].

The phenotypic variation of the nephron provides an inherent means for exploring the question of cell lineage of the nephron. Genes that are highly localized within a specific portion of the nephron can be exploited as markers to determine the embryonic cell of origin of that portion of the nephron. We used RT-PCR of four relatively well-characterized molecules to investigate the cell of origin of segments of the nephron that express these molecules. The cell of origin of renin, THP, OTR, and V2R was determined in cultured rat-mouse chimeric metanephric kidneys.

Cross-species chimeric metanephric kidneys formed by combining the ureteric bud from either the rat or mouse with the metanephric mesenchyme of the other species differentiated in culture to form developing nephrons similar to those of intact cultured metanephric kidneys. They were slower to begin differentiating, however, taking approximately 1–2 days before branching of the ureteric bud began and 3–4 days before comma- or S-shaped bodies were well developed. The chimeric kidneys were rather unorganized, lacking the stereotypical repetitive morphology of the intact explants and there were fewer glomerular structures per kidney than in intact explants. However, by confocal microscopy, the s-shaped bodies that formed were nearly indistinguishable from those formed by intact metanephric kidneys. The comma- and s-shaped bodies in the chimeric kidneys were also similar in morphology to those produced when the metanephric mesenchyme was cultured in the presence of the spinal cord.

Preparations of the metanephric mesenchyme alone or the ureteric bud alone failed to differentiate as in the chimeras, even when up to five preparations of each were cultured together. These data suggest that any residual metanephric mesenchyme cells attached to the ureteric bud, or residual ureteric bud cells remaining within the mesenchyme, were not appreciable and would not be capable of differentiating into nephron structures in the chimeras. Therefore, in the case of THP, OTR, and renin, where the mRNA for each was derived from both ureteric bud and mesenchyme cell progenitors, contaminating residual cells from one or the other component could not be responsible for elaborating these genes.

The pattern of V2R mRNA expression has been demonstrated in embryonic, postnatal, and adult rats [Ostrowski et al., 1992, 1993]. It is localized in the mature kidney to cortical and medullary collecting ducts with some expression in the TAL. During gestation (embryonic days 16 and 19), V2R is expressed only in developing collecting duct structures. In the present study, V2R mRNA was elaborated predominantly from cells that derived from the ureteric bud, in keeping with its highly restricted expression pattern. The small proportion of mRNA that appears to have derived from the metanephric mesenchyme (<20%) may be a result of minimal TAL development in the metanephric kidneys. Generally, in culture, metanephric kidneys do not develop a significant loop of Henle and this may explain the relative lack of mesenchyme-derived expression of V2R.

**Fig. 11.** Restriction digest of chimeric metanephric kidney THP PCR product. A: Polyacrylamide gel of 32P-labeled THP PCR product from UrMm and UmMr chimeras after digestion with BanII. Undigested product is 400 bp. The THP products from both of the chimera types, UrMm (61.0 ± 3.4% of total product in fragments, n = 16) and UmMr (70.9 ± 2.2% of total product in fragments, n = 14), are digested by BanII. B: Based on the restriction digestion pattern of the chimeras, the THP product was produced by both species, and therefore, must have come from both ureteric bud- and mesenchyme-derived cells.
In adult human kidneys, THP is localized to the TAL of the loop of Henle and the early portion of the DCT and is absent from late distal tubule, connecting tubule, and collecting duct [Sikri et al., 1981; Howie and Johnson, 1992]. In the embryonic kidney, the expression of THP parallels the development of the TAL and the early distal tubule [Howie et al., 1993]. In the present study, expression of THP was found to be from both ureteric bud- and mesenchyme-derived cells. These results imply that there is admixing of ureteric bud and metanephric mesenchyme cells along the midportion of the nephron, including at least the late TAL and early DCT. This region of the nephron is at the center of the area pinpointed as a possible transition zone.

In mature kidneys, renin is most highly expressed in the afferent arterioles but components of the renin-angiotensin system, including renin mRNA, are also known to be present in the proximal tubule [Taungner et al., 1982]. Renin is expressed in the vascular tree to the level of the arcuate arteries when the vasculature is at an early stage of development. As the kidney matures, the expression of renin becomes more localized to the afferent arterioles [Reddi et al., 1998]. Renin mRNA has been detected in isolated intact metanephric kidneys at 11.5 days of gestation in the mouse (Arend, unpublished results), a time at which there is no appreciable vascular development. This suggests that renin is expressed at the earliest stages of kidney development in the metanephric mesenchyme, ureteric bud, or both, possibly in vascular progenitors that reside in one of these components. While the embryonic derivation of the vasculature is controversial, some vascular elements likely originate from the metanephric mesenchyme as well as from an extrametanephric source. The results from the cross-species chimeras in the present study demonstrate that, despite the known proximal expression pattern of renin mRNA, some ureteric bud-derived cells were responsible for renin production.

The OTR is expressed in the developing rat kidney by 17 days of gestation in both medullary and cortical regions [Schmidt et al., 1990; Ostrowski et al., 1995]. In the cortex, OTR was found in tubules near glomeruli, suggesting macula densa expression. Expression was also identified in distal tubules at PND4. In the medulla, OTR expression was transiently found in the papilla/inner medulla from E17 to PND10, and later, from PND20 to PND30, during the period of weaning, only in the outer medulla. The structures expressing OTR in the medulla were felt to be loop of Henle, as collecting ducts were easily identifiable and not labeled. In mature kidneys, the OTR is exclusively in the cortex, primarily in the macula densa but with some expression in the DCT [Ostrowski et al., 1995]. In the present study, OTR mRNA expression was detected in cells that were derived from both ureteric bud- and metanephric mesenchyme-derived cells, supporting the hypothesis that there is a mixing of mesenchyme and ureteric bud cells along the nephron.

In summary, renin, THP, and OTR mRNA expression in cross-species chimeric metanephric kidneys is by cells whose progenitors were both ureteric bud and metanephric mesenchyme cells. Only V2R, which is localized to the extreme distal nephron, showed almost exclusively ureteric bud-derived expression. These results, taken together with several studies suggesting no clear transition between mesenchyme- and ureteric bud-derived cells in the nephron, indicate that the origin of each segment of the nephron may be more plastic than previously envisioned. It remains to be determined at which point in differentiation a cell is destined to become a member of a phenotypically distinct segment of the nephron.

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