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., U_rM_m and U_mM_r), 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 U_rM_m 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 certain portions of the nephron is heterogenous. Dev. Genet. 24:230–240, 1999. © 1999 Wiley-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 metanephros [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 $NaH_2PO_4 \cdot H_2O$, 140 mM NaCl, 3 mM KCl, 1.5 mM KH_2PO_4, 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% CO_2.

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 U_rM_m, while the opposite cross is designated U_mM_r.

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 Vectashield (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 DF10 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 $MgCl_2$, 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 ^{32}P dCTP (Amersham, Arlington Heights, IL), and 1–6 µl of tissue cDNA.

TABLE 1. Sequence of Primer, Resulting Amplification Product Size, and Nucleotide Position for Each Molecule

Molecule		Primer sequence	Size (bp)	Position
THP	Sense	5′ CTG GAT GTC CAT AGT GAC TC 3′		
	Antisense	5′ TGT GGC ATA GCA GTT GGT CA 3′	399	1152–1551
V2R	Sense	5′ TTT CAA GTG CTA CCC CAG CT 3′		
	Antisense	5′ AAT CGG GCC CAG CAA TCA AA 3′	316	312–628
Renin	Sense	5′ TGG GTC CCC TCC ACC AAG T 3′		
	Antisense	5′ TCC CAG GGC TTG CAT GAT CA 3′	600	540–1139
OTR	Sense	5′ AGC GTT TGG GAC GTC AAT GCG 3′		
	Antisense	5′ GGC TAT GGC TCA TGC TGA AGA TGG 3′	288	111–369
β-actin	Sense	5′ AAC CGC GAG AAG ATG ACC CAG ATC ATG TTT 3′		
	Antisense	5′ AGC AGC CGT GGC CAT CTC TTG CTC GAA GTC 3′	350	384–734

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]. *ß-actin* was used as a housekeeping gene to control for variations in sample DNA. To ensure that similar amounts of cDNA template were present between the various samples, serial 10-fold dilutions of each sample were amplified with *ß-actin* primers. After quantitation of the radioactivity, the cDNA was diluted appropriately to provide cDNA equal to that in the sample with the lowest *ß-actin* mRNA abundance. Amplifications were then performed on the *ß-actin* normalized cDNA. From each PCR reaction, the product contained in 45 µl was precipitated with 5% linear acrylamide, 4 M ammonium acetate, and 100% ethanol, resuspended in TE buffer (pH 8.0), and run on a 5% polyacrylamide gel. ^{32}P incorporation into bands was detected by autoradiography and quantitated using Phosphor Analyst software on a GS-250 Molecular Imager system (Bio-Rad).

Cloning and Sequencing of the Mouse THP and V2R PCR Products

To distinguish between the PCR products made by the mouse and rat, the nucleotide sequence of both species was needed. The rat sequence was available from the literature for both THP and V2R. The sequence of the mouse PCR product was determined by TA cloning and sequencing of the PCR product. Briefly, the PCR products were ligated into the pCR vector (Invitrogen Corp., San Diego, CA) and competent bacteria were transformed with the plasmid. Positive colonies were picked and grown in LB media containing ampicillin. Mini-prep DNA (Qiagen, Chatsworth, CA)

from overnight cultures of the clones was subjected to restriction digestion with *Eco*RI to verify the presence of an appropriate sized insert. Plasmid DNA was sequenced on an ABI 373A autosequencer. Sequences were compared to known sequences of other species by the Blast program of the National Center for Biotechnology Information.

Restriction Enzyme Digestion of PCR Products

PCR products were precipitated as described above and resuspended in dH$_2$O. 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
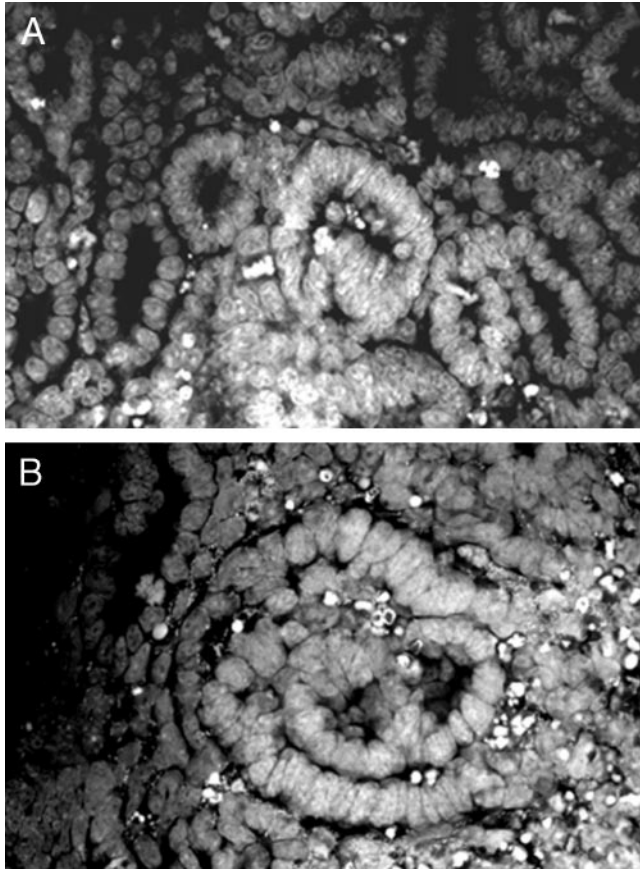
Fig. 1. Confocal microscopic images of a chimeric metanephric kidney composed of ureteric bud from an 11.5 day gestation mouse and metanephric mesenchyme from a 13.5 day gestation rat (U_mM_r) after 7 days in culture. **A:** Comma-shaped body and developing tubules. **B:** S-shaped body. Nuclei are visualized with propidium iodide.

Fig. 2. Restriction digestion of renin PCR product from intact mouse and rat cultured metanephric kidneys. **A:** Expected digestion pattern of mouse and rat renin product by *Eco*RI and *Bst*XI based on restriction maps for each species. Undigested product is 600 bp. *Eco*RI is expected to cleave only rat renin PCR product into 100 and 500 bp fragments, while *Bst*XI should cut mouse product into 85 and 515 bp fragments. **B:** Renin PCR product from intact mouse and rat cultured metanephric kidneys digested with *Eco*RI and *Bst*XI. The rat product is cut by *Eco*RI and the mouse product is cut by *Bst*XI as expected from the restriction maps.

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 *Eco*RI and *Bst*XI 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, *Eco*RI cut the rat product (600 bp uncut) into two fragments of 100 and 500 bp, but did not digest the mouse product. *Bst*XI 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 *Eco*RI and *Bst*XI.
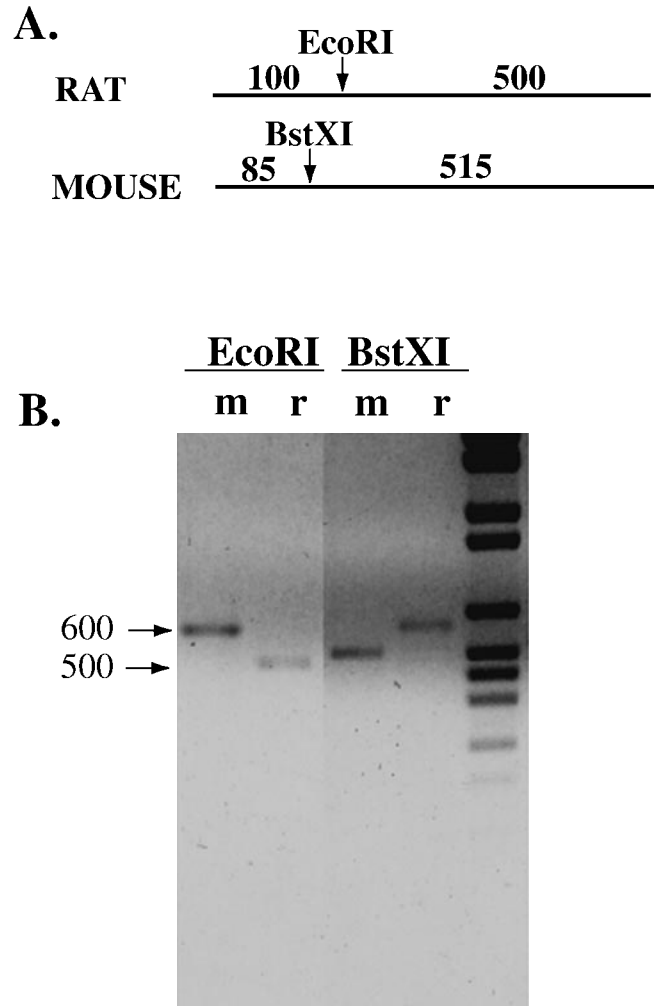
The PCR product from both chimeras (Fig. 3) was digested equally well by both enzymes. By densitometry, $53.9 \pm 3.8\%$ of the renin product from U_rM_m chimeras (n = 8) was found in the restriction fragments when *Eco*RI was used and $54.1 \pm 5.1\%$ was digested when *Bst*XI was used. Similar results were found with the U_mM_r chimeras (n = 4). When *Eco*RI was used to digest the U_mM_r chimeras, $52.7 \pm 6.5\%$ of the product was found in the fragments, while *Bst*XI digestion of the renin product from U_mM_r chimeras resulted in $55.9 \pm 1.8\%$ of the product in fragments. These results indicate that the renin mRNA present was produced by
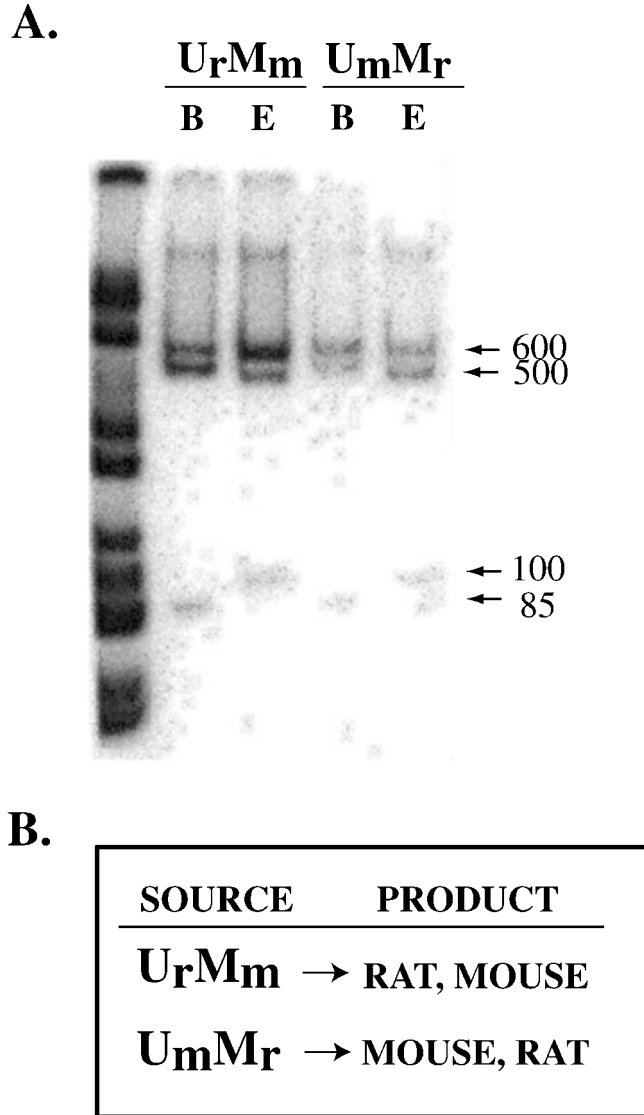
A.

A.

B.

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 *Ban*I and *Nci*I based on restriction maps for each species. Undigested product size is 288 bp. *Ban*I would be expected to cleave only mouse OTR PCR product into 129 and 159 bp fragments, while *Nci*I 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 *Ban*I and *Nci*I. The mouse product is cut by *Ban*I and the rat product is cut by *Nci*I as expected from the restriction maps.

B.

Fig. 3. Restriction digestion of chimeric metanephric kidney renin PCR product. **A:** Autoradiogram of ^{32}P-labeled renin PCR product from representative U_rM_m and U_mM_r chimeras after digestion with *Eco*RI and *Bst*XI. Undigested product is 600 bp. Approximately 55% of the renin product from either type of chimera was found in the fragments when either *Bst*XI (B) or *Eco*RI (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.

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 *Ban*I and *Nci*I 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
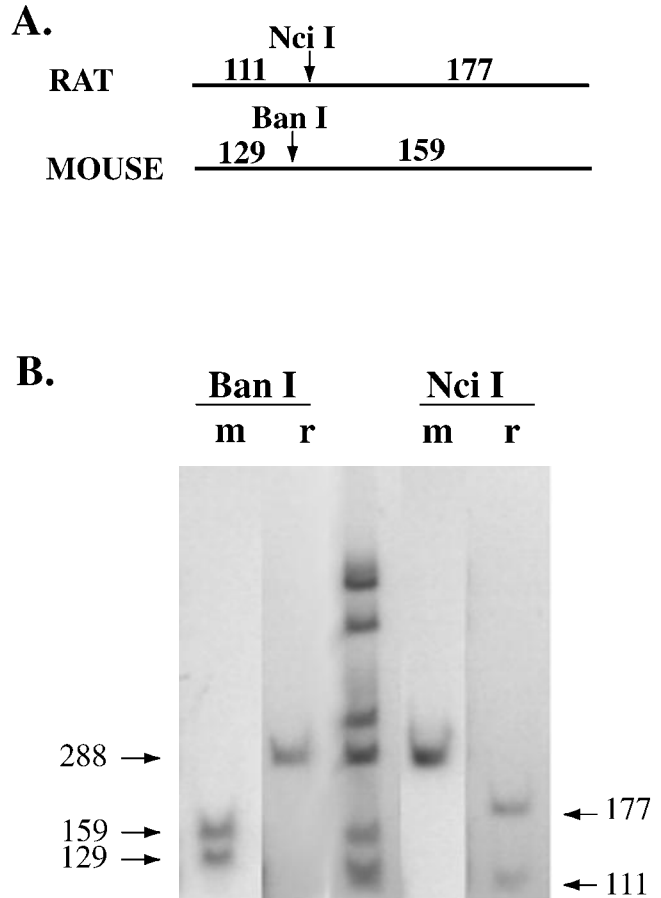
from either mouse or rat intact cultured metanephric kidneys are shown in Figure 4.

As expected, *Ban*I cut the mouse product (288 bp uncut) into two fragments of 129 and 159 bp (Fig. 4B), but did not digest the rat product. *Nci*I 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 *Ban*I and *Nci*I. The PCR product from both chimeras (Fig. 5A) was digested equally well by both enzymes. By densitometry, $60.6 \pm 3.6\%$ of the OTR product from U_rM_m

A.

U_rM_m

Ban I   Nci I

U_mM_r

Ban I   Nci I

288 →

159 →
129 →

← 177

← 111

B.
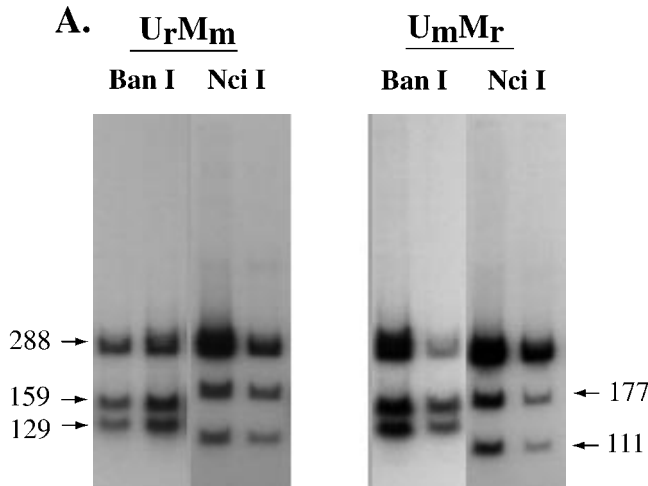
SOURCE	PRODUCT
U_rM_m →	RAT, MOUSE
U_mM_r →	MOUSE, RAT

Fig. 5. Restriction digestion of chimeric metanephric kidney OTR PCR product. **A:** OTR PCR product from representative U_rM_m and U_mM_r chimeras after digestion with *Ban*I and *Nci*I. Undigested product is 288 bp. Approximately 55–60% (see Results) of the OTR product from either type of chimera was found in the fragments when either *Ban*I or *Nci*I was used. **B:** In either chimeric metanephric kidney type, both the mouse and rat contributed to the OTR product, suggesting the cellular derivation was both metanephric mesenchyme and ureteric bud.

chimeras (n = 13) was found in the restriction fragments when *Ban*I was used and 55.9 ± 3.7% was digested when *Nci*I was used. Similar results were found with the U_mM_r chimeras (n = 16). When *Ban*I was used to digest the U_mM_r chimeras, 62.9 ± 1.9% of the product was found in the fragments, whereas *Nci*I digestion of the OTR product from U_mM_r 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 [Lolait *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.
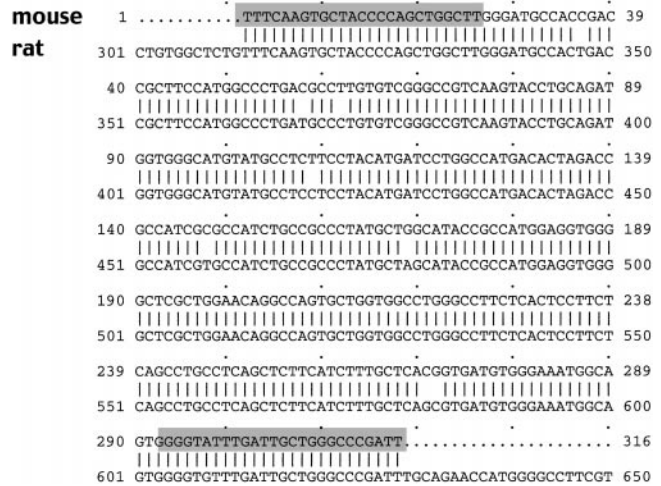

Fig. 6. Sequence of mouse V2R PCR product (upper row) aligned with known rat sequence (lower row). Shaded areas represent regions used as forward and reverse PCR primers. Total amplification product size: 316 bp. Vertical bars indicate nucleotide identity between species (97%).

Restriction Digestion of the V2R PCR Product

The restriction enzyme *Nhe*I was chosen to differentiate between mouse and rat V2R PCR products based on the restriction maps for each species (Fig. 7A). Results of *Nhe*I digestion of the V2R product from intact mouse and rat cultured metanephric kidneys are shown in Figure 7B. *Nhe*I 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 *Nhe*I. The PCR product from chimeras of the U_rM_m type (Fig. 8, left panel; n = 7) was digested by *Nhe*I 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_rM_m chimeras was found in the restriction fragments. These results are consistent with product derived predominantly from the rat portion of the U_rM_m chimera, i.e., the ureteric bud. Alternatively, 33.4 ± 7.8% of the V2R product from U_mM_r chimeras (Fig. 8, right panel; n = 9) was found in the restriction fragments, leaving nearly 70% uncut, 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 *Nhe*I consistently demonstrated that the V2R mRNA present in these chimeras predominantly derived from the ureteric bud, rather than from the metanephric mesenchyme.
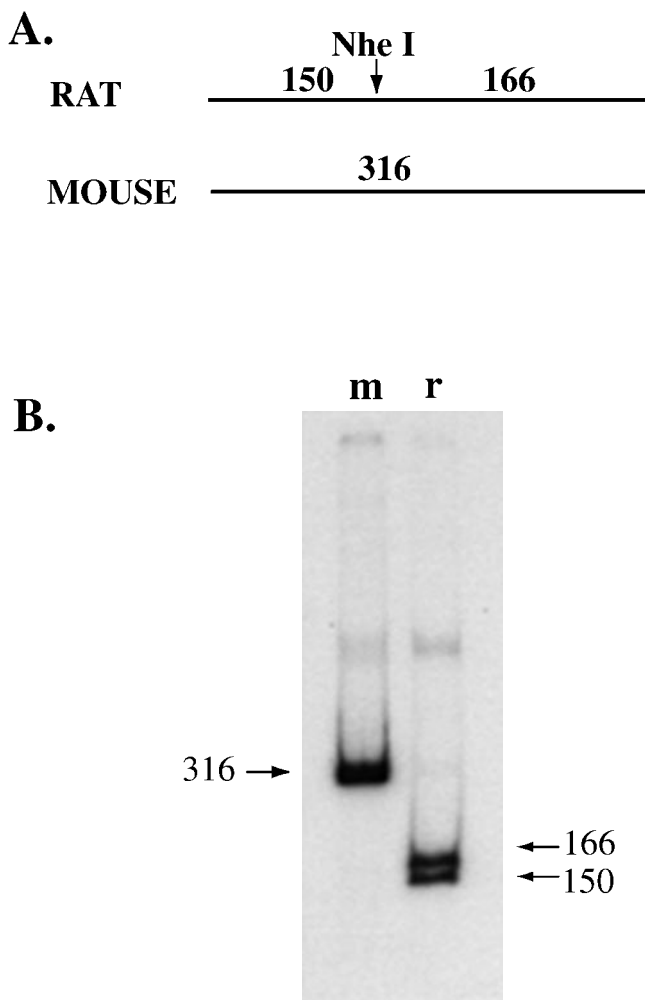
A.

Nhe I

RAT   150   ↓   166

MOUSE   316

B.

m   r

316 →

←166
←150

Fig. 7. Restriction digest of V2R PCR product from mouse and rat cultured metanephric kidneys. **A:** Expected digestion pattern of mouse and rat V2R product by *Nhe*I based on restriction maps of the PCR product from each species. Undigested product is 316 bp. *Nhe*I should cleave rat to produce 166 and 150 bp fragments. *Nhe*I should not digest mouse V2R product. **B:** Autoradiogram of mouse and rat V2R PCR products digested with *Nhe*I, producing the expected restriction fragment pattern; only the rat product is cleaved by *Nhe*I.

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 *Ban*II was chosen to differentiate between mouse and rat THP PCR product
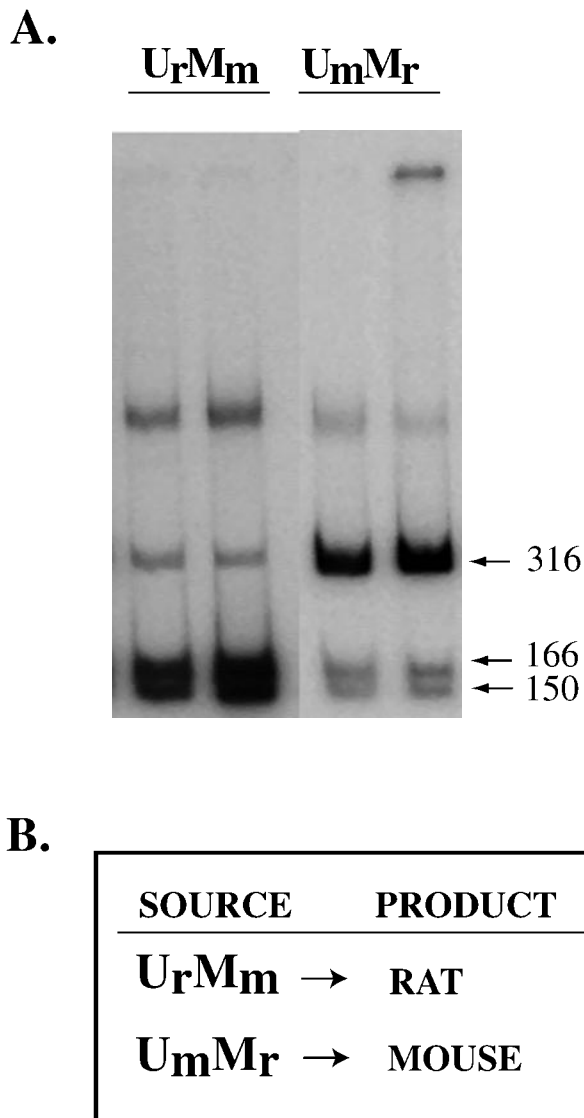
A.

U_rM_m U_mM_r

← 316

← 166
← 150

B.

SOURCE	PRODUCT
U_rM_m →	RAT
U_mM_r →	MOUSE

Fig. 8. Restriction digest of chimeric metanephric kidney V2R PCR products. **A:** Autoradiogram of ^{32}P-labeled V2R PCR product from U_rM_m and U_mM_r chimeras after digestion with *Nhe*I. Undigested product is 316 bp. The V2R PCR product from the U_rM_m chimera is digested by *Nhe*I (77.3 ± 7.6% of total product in fragments, n = 7) but the product from the U_mM_r chimera was not cut (33.4 ± 7.8% of total product in fragments, n = 9). **B:** The species producing the V2R product in the U_rM_m chimera, based on the restriction digest pattern, is rat. In the U_mM_r chimera, mouse tissue produced the V2R. In each case, the V2R was produced by the species that provided the ureteric bud.

(Fig. 10A). Results of *Ban*II digestion of the THP product from intact mouse and rat cultured metanephric kidneys are shown in Figure 10B. *Ban*II cleaved the THP product from the mouse into 270 and 130 bp fragments, but as expected, did not cut rat product (400 bp).
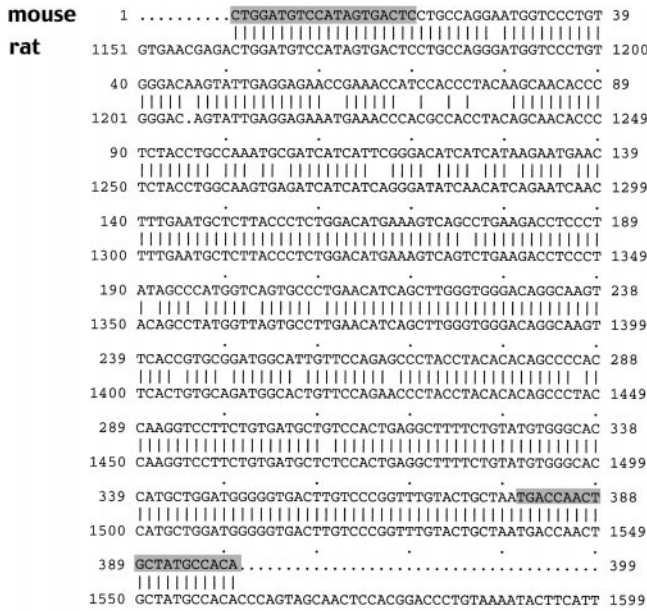
```
mouse     1 ..........CTGGATGTCCATAGTGACTCCTGCCAGGAATGGTCCCTGT 39
             |||||||||||||||||||||||||||||| |||||||||||
rat    1151 GTGAACGAGACTGGATGTCCATAGTGACTCCTGCCAGGGATGGTCCCTGT 1200

         40 GGGACAAGTATTGAGGAGAACCGAAACCATCCACCCTACAAGCAACACCC 89
             |||||  |||||| ||||||||||  ||||||  |||      ||||||||
rat    1201 GGGAC.AGTATTGAGGAGAAATGAAACCCACGCCACCTACAGCAACACCC 1249

         90 TCTACCTGCCAAATGCGATCATCATTCGGGACATCATCATAAGAATGAAC 139
             ||||||| ||| || ||||||||||  |||| ||||  ||| ||||| |||
rat    1250 TCTACCTGGCAAGTGAGATCATCATCAGGGGATATCAACATCGGAATCAAC 1299

        140 TTTGAATGCTCTTACCCTCTGGACATGAAAGTCAGCCTGAAGACCTCCCT 189
             |||||||||||||||||||||||||||||||||| ||||||||||||||||
rat    1300 TTTGAATGCTCTTACCCTCTGGACATGAAAGTCAGTCTGAAGACCTCCCT 1349

        190 ATAGCCCATGGTCAGTGCCCTGAACATCAGCTTGGGTGGGACAGGCAAGT 238
             | ||||  ||||||| ||||||||||||||||| |||||||||||||||||
rat    1350 ACAGCCTATGGTTAGTGCCTTGAACATCAGCTTGGGTGGGACAGGCAAGT 1399

        239 TCACCGTGCGGATGGCATTGTTCCAGAGCCCTACCTACACACAGCCCCAC 288
             ||||  |||| |||||||| ||||||||| ||||||||||||||||||  |
rat    1400 TCACTGTGCAGATGGCACTGTTCCAGAACCCTACCTACACACAGCCCTAC 1449

        289 CAAGGTCCTTCTGTGATGCTGTCCACTGAGGCTTTTCTGTATGTGGGCAC 338
             |||||||||||||||||||||| ||||||||||||||||||||||||||||
rat    1450 CAAGGTCCTTCTGTGATGCTCTCCACTGAGGCTTTTCTGTATGTGGGCAC 1499

        339 CATGCTGGATGGGGGTGACTTGTCCCGGTTTGTACTGCTAATGACCAACT 388
             ||||||||||||||||||||||||||||||||||||||||||
rat    1500 CATGCTGGATGGGGGTGACTTGTCCCGGTTTGTACTGCTAATGACCAACT 1549

        389 GCTATGCCACA....................................... 399
             |||||||||||
rat    1550 GCTATGCCACACACCCAGTAGCAACTCCACGGACCCTGTAAAATACTTCATT 1599
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Fig. 9. Sequence of mouse THP PCR product (upper row), aligned with known rat sequence (lower row). Shaded areas represent regions used as forward and reverse PCR primers. Total amplification product size: 400 bp. Vertical bars indicate nucleotide identity between species (92%).

Origin of THP PCR Product From Chimeric Metanephric Kidneys

THP PCR product from cultured chimeric kidneys was subjected to restriction digestion with *Ban*II. THP product from chimeras of both types (Fig. 11A) was digested by *Ban*II to a similar degree. By densitometry, $61 \pm 3.4\%$ of the THP product from U_rM_m chimeras (n = 16) was found in the restriction fragments and $70.9 \pm 2.2\%$ of the THP product from U_mM_r chimeras (n = 14) was cut by *Ban*II. Since *Ban*II 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,
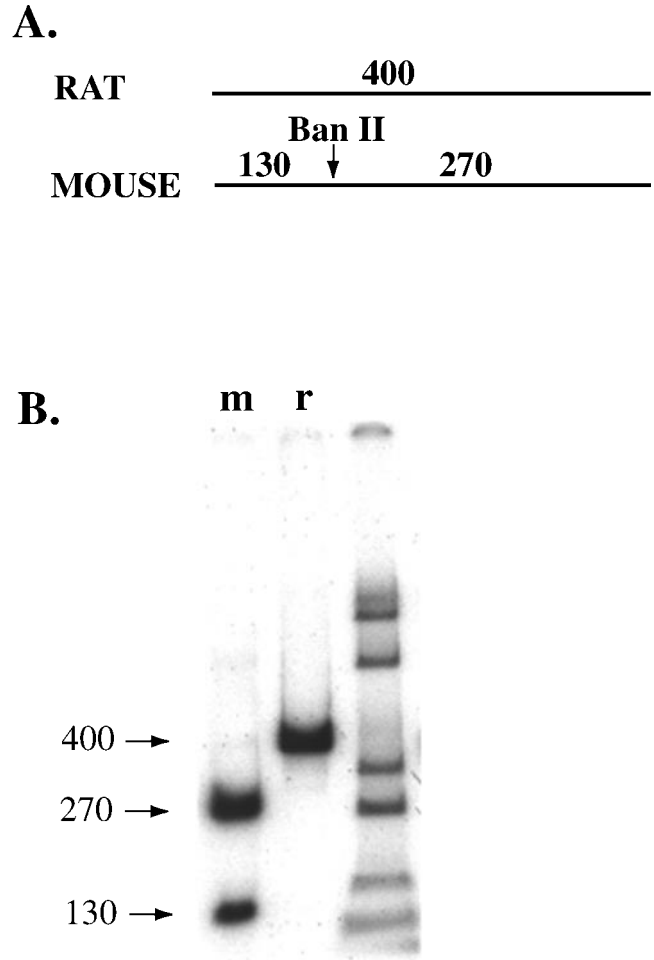
A.

B.

Fig. 10. Restriction digest of THP PCR product from mouse and rat metanephric kidneys. **A:** Expected digestion pattern of mouse and rat THP product by *Ban*II. Undigested product is 400 bp. *Ban*II should cleave mouse THP to produce 270 and 130 bp fragments but should not digest the rat THP product based on restriction maps of the PCR product from each species. **B:** Autoradiogram of mouse and rat THP PCR products digested with *Ban*II, producing the expected restriction fragment pattern; only the mouse product is cleaved by *Ban*II.

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-

A.

$$U_rM_m \quad U_mM_r$$

400 →

270 →

130 →

B.
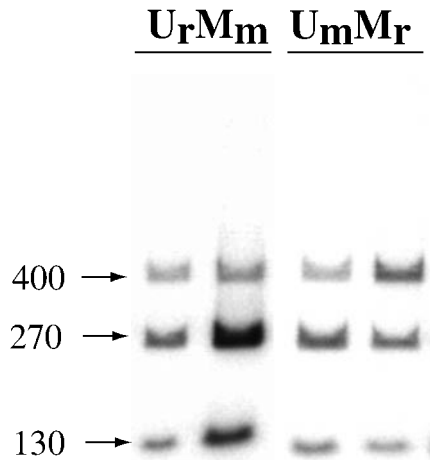
SOURCE	PRODUCT
U_rM_m →	RAT, MOUSE
U_mM_r →	MOUSE, RAT

Fig. 11. Restriction digest of chimeric metanephric kidney THP PCR product. **A:** Polyacrylamide gel of ^{32}P-labeled THP PCR product from U_rM_m and U_mM_r chimeras after digestion with *Ban*II. Undigested product is 400 bp. The THP products from both of the chimera types, U_rM_m (61.0 ± 3.4% of total product in fragments, n = 16) and U_mM_r (70.9 ± 2.2% of total product in fragments, n = 14), are digested by *Ban*II. **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.

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.

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 [Taugner *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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