Developmental Biology **246,** 296–310 (2002)
doi:10.1006/dbio.2002.0646

View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Bud and by the Renal Stroma

Jun Yang,* Alexander Blum,* Thaddeus Novak,* Randy Levinson,† Eseng Lai,† and Jonathan Barasch*,[1]

**College of Physicians and Surgeons of Columbia University, and †Memorial Sloan Kettering Cancer Center, New York, New York 10032*

Kidney epithelia develop from the metanephric mesenchyme after receiving inductive signals from the ureteric bud and from the renal stroma. However, it is not clear how these signals induce the different types of epithelia that make up the nephron. To investigate inductive signaling, we have isolated clusters of epithelial progenitors from the metanephric mesenchyme, thereby separating them from the renal stroma. When the isolated progenitors were treated with the ureteric bud factor LIF, they expressed epithelial proteins (ZO-1, E-cadherin, laminin α_5) and produced nephrons (36 glomeruli with 58 tubules), indicating that they are the target of inductive signaling from the ureteric bud, and that renal stroma is not absolutely required for epithelial development *in vitro*. In fact, stroma-depleted epithelial progenitors produced sevenfold more glomeruli than did intact metanephric mesenchyme (5 glomeruli, 127 tubules). Conversely, when epithelial progenitors were treated with both LIF and proteins secreted from a renal stromal cell line, glomerulogenesis was abolished but tubular epithelia were expanded (0 glomeruli, 47 tubules). Hence, by isolating epithelial progenitors from the metanephric mesenchyme, we show that they are targeted by factors from the ureteric bud and from the renal stroma, and that epithelial diversification is stimulated by the ureteric bud and limited by renal stroma. © 2002 Elsevier Science (USA)

Key Words: **epithelia; progenitor; mesenchyme; induction; kidney; stroma.**

INTRODUCTION

The nephron, the functional unit of the kidney, is an epithelial tubule that derives from metanephric mesenchyme. Many types of epithelia make up the nephron, including glomerular and tubular epithelia. All of these epithelia are thought to arise from a multipotent progenitor (Herzlinger *et al.,* 1992). Although this cell has not been directly identified, we know that it (or its daughters) must express the transcription factors WT-1 (Kreidberg *et al.,* 1993) and Pax-2 (Rothenpieler and Dressler, 1993) and the signaling molecule Wnt-4 (Vainio and Muller, 1997; Stark *et al.,* 1994) in order to epithelialize.

Cell–cell signaling is an important mechanism that regulates epithelial conversion. Mesenchyme converts to epithelia only after it has been invaded by an outgrowth of the Wolffian duct, called the ureteric bud (Grobstein, 1955; Saxen, 1987; Schuchardt *et al.,* 1994; reviewed in Vainio and Muller, 1997; Barasch, 2001). Yet the specific role of the ureteric bud remains obscure because its signaling molecules have not been identified. For example, it is not clear whether the ureteric bud regulates a multipotent progenitor or its daughters, or whether it stimulates the formation of one or more types of epithelia that are found in the nephron.

In addition to ureteric bud, renal stroma is thought to regulate the epithelial lineage. Deletion of the transcription factor Foxd1/BF-2, which is specifically expressed in a subset of renal stroma (Hatini *et al.,* 1996), reduces the number of fully formed nephrons. Kidneys from these animals accumulate Pax-2-, WT-1-, and Wnt-4-expressing cells, suggesting that they require renal stroma to progress to epithelia. However, the precise function of the renal stroma in the development of epithelia is not known.

To determine how epithelial progenitors give rise to the many types of epithelia of the nephron, it is necessary to examine how signaling from the ureteric bud and from the renal stroma regulates conversion. The best experimental approach is to isolate epithelial progenitors and expose them to factors produced by the ureteric bud and by the renal stroma. This approach is necessary because, while the ureteric bud and the renal stroma may regulate epithelial

[1] To whom correspondence should be addressed. Fax: (212) 305-3475. E-mail: jmb4@columbia.edu.

0012-1606/02 $35.00
© 2002 Elsevier Science (USA)
All rights reserved.

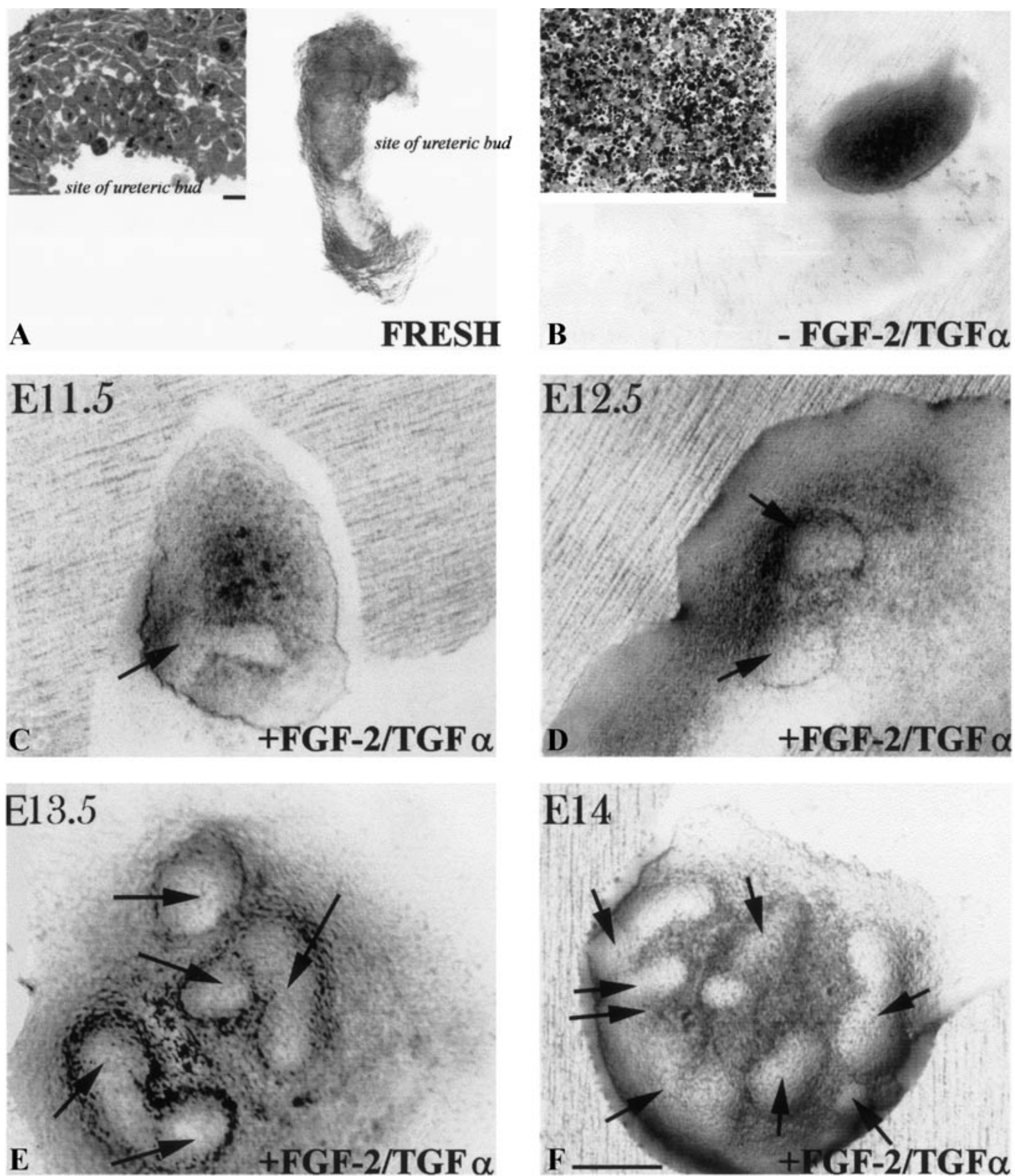


FIG. 1. Development of cellular clusters of epithelial progenitors in cultured metanephric mesenchyme. (A) Metanephric mesenchyme, freshly isolated from its ureteric bud. Lateral view and frontal section through the isolated metanephric mesenchyme (inset) shows the cavity where the ureteric bud had been ("site of the ureteric bud"). (B) When incubated in basal media for over 24 h, isolated metanephric mesenchyme undergoes apoptosis. The inset is a section of the mesenchyme showing prominent apoptotic bodies. The entire tissue will involute and disappear by 48 h of culture. (C–F) Metanephric mesenchyme cultured with FGF-2/TGFα is viable and forms cellular clusters. The number of these cellular clusters is proportional to the age of the donor. (C) Metanephric mesenchyme from the down-growing Wolffian duct of the $E_{11.5}$ embryo. (D) Metanephric mesenchyme from the unbranched ureteric bud at $E_{12.5}$, and (E) from a singly branched ureteric bud at $E_{13.5}$. (F) Metanephric mesenchyme from the twice branched ureteric bud at E_{14}. Bars, 250 μm; insets, 10 μm.

conversion, the mesenchyme in turn regulates the development of the ureteric bud and the stroma (Saxen, 1987; Vainio and Muller, 1997; Dudley *et al.,* 1999; reviewed in Schedl and Hastie, 2000). By isolating epithelial progenitors, we can analyze cell conversion without concern for the reciprocal effect of these cells on the development of the ureteric bud and the renal stroma.

In the current work, we have isolated and characterized a single population of epithelial progenitors. We have examined the actions of the ureteric bud and renal stroma in

© 2002 Elsevier Science (USA). All rights reserved.

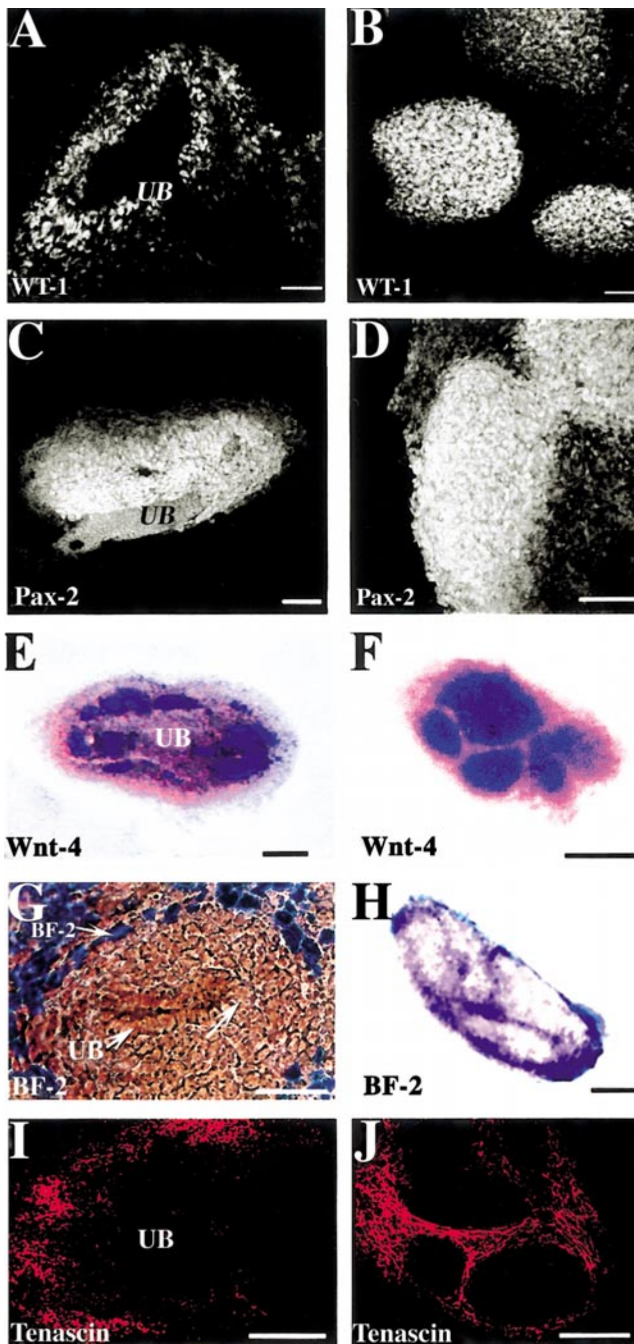


FIG. 2. Mesenchymal cells surrounding the ureteric bud *in vivo* express the same regulatory proteins as the cellular clusters *in vitro*. WT-1 (A) and Pax-2 (B) were detected by immunocytochemistry and Wnt-4 (C) by *in situ* hybridization in cells surrounding the ureteric buds of $E_{13.5}$ rat kidneys and in the cellular clusters of metanephric mesenchyme placed in culture for 24 h with FGF-2/TGFα (B, D, F). In contrast, genes that mark renal stroma were expressed in the periphery of kidneys and metanephric mesenchyme *in vitro*. BF-2/Foxd1 gene (G) is shown in $E_{11.5}$ mouse kidneys by the expression of β-galactosidase in the BF-2 locus (Hatini *et al.*, 1996) and tenascin protein (I) is detected by immunocytochemistry in $E_{13.5}$ rat kidneys. BF-2/Foxd1 (H) and tenascin (J) in cultured metanephric mesenchyme surround the cellular clusters, similar to their peripheral distribution in developing kidney. UB, ureteric bud. (A, G) Sections; others are whole mounts. Bars, (A, C) 300 μm; (E, G, I) 200 μm; (B, D) 100 μm; (F, H, J) 250 μm.

their conversion to epithelia. We first found these cells by noting distinct cellular clusters in the metanephric mesenchyme during *in vitro* organ culture (Barasch *et al.*, 1999b). These progenitors produce many types of epithelia when they are treated with LIF, an inducer of epithelia that is specifically expressed by the ureteric bud (Barasch *et al.*, 1999b; Plisov *et al.*, 2001). The progenitors produced diverse epithelia even when they were isolated from renal stromal cells (prior to the addition of LIF), demonstrating that stroma, unlike the ureteric bud, is not strictly required for epithelial conversion *in vitro*. In fact, the isolated cellular clusters of epithelial progenitors generated sevenfold more glomeruli than when contacted by renal stroma in the metanephric mesenchyme. The shift in epithelial cell type was due to the removal of stromal cells from epithelial progenitors, because readdition of Foxd1/BF-2$^+$ stromal cells or factors secreted by these cells abolished glomerulogenesis. Hence, a combination of factors from the ureteric bud and from the renal stroma determine epithelial type. The stroma modifies the types of epithelia induced by the ureteric bud.

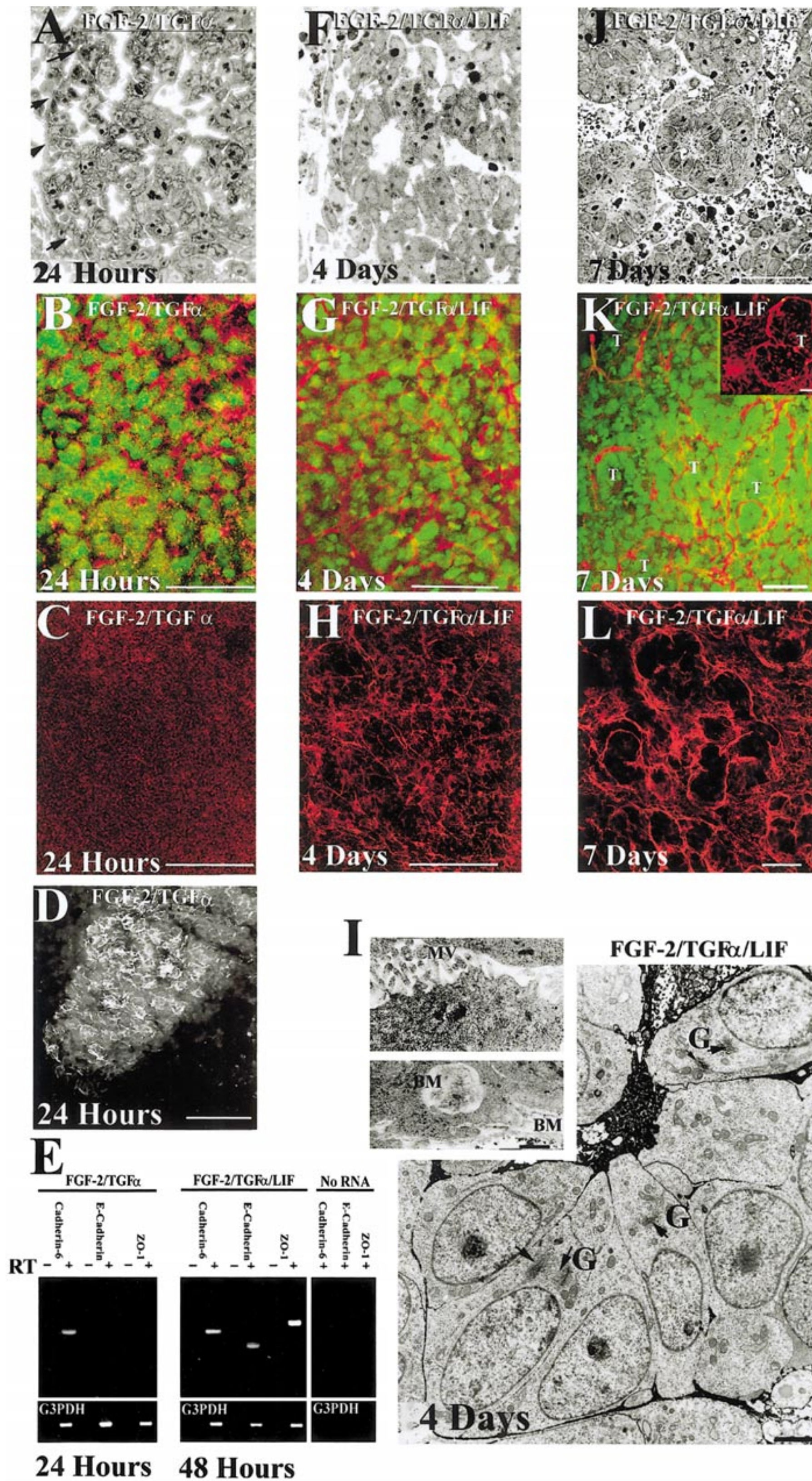
METHODS

Identification and Isolation of an Epithelial Precursor

Rat $E_{11.5}$–E_{14} metanephric mesenchyme was isolated from its ureteric bud by treating embryonic kidneys with DNase (100 U/ml; Boehringer-Mannheim) and trypsin (1 mg/ml; Sigma) for 15 min at 37°C in L-15 media (Herzlinger *et al.*, 1992; Barasch *et al.*, 1996), and then freeing the mesenchyme with minutia pins (Fine Scientific). The mesenchyme was then washed in L-15 with soybean trypsin inhibitor (1 mg/ml). Contamination by fragments of ureteric bud was ruled out by the absence of dolichos bifloris binding sites and epithelial proteins (e.g., E-cadherin, cytokeratin, laminin α_5), which are only expressed by the ureteric bud at this stage (Vestweber *et al.*, 1985; Miner *et al.*, 1997; Barasch *et al.*, 1996, 1997, 1999a,b; Plisov *et al.*, 2001). In addition, the isolated cells undergo fulminant apoptosis, which requires the absence of even small fragments of the ureteric bud (Koseki *et al.*, 1992; Barasch *et al.*, 1997). The ureteric bud can be removed with ease up to E_{14}.

Isolated metanephric mesenchyme was cultured on transwell filters (Costar) at an air-fluid interface in serum-free media with FGF-2 (50 ng/ml) and TGFα (10 ng/ml) (FGF-2/TGFα; R&D Systems) according to Karavanova *et al.* (1996) (Barasch *et al.*, 1997, 1999b). FGF-2/TGFα prevents apoptosis and maintains cells that are competent to undergo cell conversion (Herzlinger *et al.*, 1992; Karavanova *et al.*, 1996; Barasch *et al.*, 1997). Moreover, these

© 2002 Elsevier Science (USA). All rights reserved.



© 2002 Elsevier Science (USA). All rights reserved.

cultured metanephric mesenchymes developed visible cellular clusters that could be pushed out of the body of the mesenchyme with a dissecting needle. The ease of the removal of these cells from the metanephric mesenchyme may be the result of their expression of cell–cell adhesion proteins and their limited interactions with neighboring cells. Isolated cellular clusters were then treated with collagenase (2 mg/ml; Worthington) in DMEM at 37°C for 15 min to remove the external cell layer. The clusters were then washed three times in fresh DMEM and placed on a transwell filter (Costar 3425) in serum-free DMEM/F-12 media with FGF-2/TGFα (see Karavanova *et al.,* 1996; Barasch *et al.,* 1999a,b). To assay for the presence of stroma, we used antibodies to tenascin for immunocytochemistry (ICC) and immunoblot (IB) (1:400 ICC, 1:1000 IBB; Chemicon AB1951), *in situ* for BF-2/Foxd1 (Hatini *et al.,* 1996), and expression of LacZ driven by the Foxd1/BF-2 promoter (Hatini *et al.,* 1996). To assay for endothelial cells, we used anti-von Willebrand factor (Barasch *et al.,* 1997).

To induce epithelial development in the metanephric mesenchyme or in the extracted cellular clusters, we added LIF (20 ng/ml) and FGF-2/TGFα in serum-free media (Karavanova *et al.,* 1996; Barasch *et al.,* 1999b) and cultured for 7 days without changing the media. To characterize the conversion, we used antibodies to NCAM (1:200 ICC, 1:1000 IB; Sigma) (Klein *et al.,* 1988), WT-1 (1:100 ICC, 1:200 IB; Santa Cruz C-192), Pax-2 (1:200 ICC, 1:1000 IB; Zymed 71-6000) (Dressler and Douglass, 1992), cadherin-6 (Cho *et al.,* 1998), collagen IV (1:2000 ICC; Biodesign T40263R), laminin α5 (1:400 ICC) (Miner *et al.,* 1997; Miner and Li, 2000), podocalyxin (1:500 ICC, 1:5000 IB) (Kerjaschki *et al.,* 1984), E-cadherin (1:50 ICC, 1:2500 IB; Transduction Labs), ZO-1 (1:200 ICC, 1:1500 IB; Zymed), a lectin specific for neuraminidase-treated glomeruli (50 μg/ml, peanut lectin; Vector) (Laitinen *et al.,* 1987), and one for proximal tubules (50 μg/ml, lotus tetraglobinus; Vector) (Laitinen *et al.,* 1987). Nuclei were visualized with Sytox (Molecular Probes). All antibodies were validated by immunoblot using E_{16} rat kidney. Last, morphology of the converting cells was examined by sections of Epon-embedded tissue stained with Toluidine Blue and by electron microscopy from lanthanum and osmium-treated samples, according to Neaves (1973).

A proliferative response to LIF was examined by the incorporation of 5′ bromodeoxyuridine (BrdU) into the cellular clusters. Metanephric mesenchyme or the extracted cellular clusters were cultured overnight with FGF-2/TGFα or with FGF-2/TGFα + LIF (as above) and then pulsed with BrdU (5 μm, 1 h). The samples were then washed three times and fixed with 4% paraformaldehyde, and BrdU was detected according to the manufacturer (Amersham).

We also characterized the conversion with *in situ* hybridization for Wnt-4 (Stark *et al.,* 1994) and RT-PCR using RNAzol B-extracted RNA (Tel-Test) followed by purification of the poly(A) fraction with Oligo-Tex Kits (Qiagen). Poly(A) RNA prepared from each experimental condition was equally divided (0.25 μg/reaction) into a series of HotStart-50 tubes (MβP), reverse transcribed with the GeneAmp kit (Perkin-Elmer), and then tested for the expression of a number of genes with primers designed by the GCG-Prime Program (Madison, WI) and 35 cycles (cadherin-6, forward: 5′-CCCTACCCAACTTTCTCAAAC, reverse: 5′-TTTCTCTTC-CCTGTCCAGCC; product 318 at 59°C annealing temperature; E-cadherin, forward: 5′-GGAAGTGATTCGAAATGATGTG, reverse: 5′-TCAGAACCACTCCCCTCATAG; product 183 at 57°C annealing temperature; ZO-1, forward: TCAGATCCCTGTAAGT-CACC, reverse: CCATCTCTTGCTGCCAAAC; product 327 at 56°C annealing temperature). The quality of the poly(A) RNA and the reverse transcription was verified by using a G3PDH internal control kit for PCR (Clontech) for every reaction. The annealing temperature was 60°C and the product was 450 bp. PCR controls also included using the identical sample of poly(A) RNA with the same primers for PCR using the HotStart and GeneAmp kits, but without adding the reverse transcriptase enzyme (−RT). In addition, the identical RT-PCR with reverse transcription but without a poly(A) RNA template was performed (−RNA). Last, every PCR product was confirmed by sequencing after isolation with the Qiaex II kit (Qiagen) or by using a second primer.

Epithelial conversion was also followed by labeling single cells with Ψ_2 retrovirus carrying β-galactosidase (Cepko *et al.,* 2000). First, viral stock solutions were prepared from the Ψ_2 BAG α cell line (ATCC) and contamination with helper virus excluded according to the manufacturer's instructions. To determine the titer of the stock solution, isolated cellular clusters were exposed to a serial dilution for 3 h in DMEM, 10% FCS + polybrene (16 μg/ml), following the procedure of Herzlinger *et al.* (1992). The infected clusters were then cultured with FGF-2/TGFα + LIF in serum-free media for 48 h and then fixed and processed for β-galactosidase histochemistry (Herzlinger *et al.,* 1992). The infected (dark blue) cells were then counted. To follow the development of a single cell, the cellular clusters were infected with an amount of virus that led to the labeling of no more than a single cell/cellular cluster (as determined by the serial dilution series; see Results) followed by culture for 7 days with LIF (20 ng/ml) and FGF-2/TGFα. The labeled colony was then examined by using serial sections from Epon-embedded cellular clusters.

FIG. 3. Epithelialization of the cellular clusters in cultured metanephric mesenchyme after the addition of the ureteric bud inducer, LIF. (A–E) Metanephric mesenchyme cultured for 24 h with FGF-2/TGFα, without LIF. (A) The cell clusters (the boundary of the cluster is indicated by arrows) contain densely packed cells that (B) express collagen IV in a disorganized pattern (collagen IV, red; nuclear sytox stain, green), but lack expression of laminin α5 (C). The predominant cell adhesion molecule is NCAM (D), but cadherin-6 (E) can be detected by RT-PCR. (E–L) Metanephric mesenchyme cultured for 2–7 days with FGF-2/TGFα + LIF (20 ng/ml). (E) Within 48 h of adding LIF, cells express E-cadherin and ZO-1; no expression was seen without LIF. [RT-PCR: without reverse transcriptase (−); with reverse transcriptase (+); "No RNA" is the complete RT-PCR without template; G3PDH is a control amplification]. (F–I) Four days after adding LIF. (F) The cells in the clusters have produced small chains that are associated with (G) strips of collagen IV$^+$ basement membrane. (H) In addition, there is *de novo* expression of laminin α5. (I) Epithelial polarity is now detectable by electron microscopy. The cells have a basement membrane (inset, BM), basal nuclei, supranuclear golgi (arrow, g), and free apical membranes with specializations resembling microvilli (MV), indicating the initiation of epithelial polarity (electron dense lanthanum counterstain shows the boundary of this chain of cells). (J–L) Seven days after exposure to LIF, (J) the cellular clusters have formed tubules and early nephrons. (K) The nascent epithelia are surrounded by organized basement membranes of collagen IV (T, tubule; inset, C-shaped nephron) and (L) laminin α5. Bars, (A–C, F–H, I) 2 μm; (J–L and K inset) 100 μm; (D) 75 μm.

© 2002 Elsevier Science (USA). All rights reserved.

Cytokine Signaling

Activation of second messengers was detected by immunoblot for phosphotyrosine[705] STAT-3 or "total" STAT-3 (Cell Signaling). The suppressors of cytokine signaling were detected by RT-PCR at 35 cycles using *Socs1* forward: 5′-TGGCACGCATCCCTCT-TAAC, reverse: 5′-AAGGTCTCCAGCCAGAAGTG; product length 225 at 56°C annealing temperature; *Socs2* forward: 5′-ATTAGAGATAGCTCCCACTCAG, reverse: 5′-GTAAAGG-TAGTCCCCGGATG; product length 337 at 54°C annealing temperature; *Socs3* forward: 5′-GTACCCCCAAGAGAGCTTAC, reverse: 5′-TTAAAGTGGAGCATCATACTGG; product length 203 at 55°C annealing temperature; *CIS* forward: 5′-ATG-AACCGAAGGTGCTAGAC, reverse: 5′-TAATGCTGCACAAG-GCTGAC; product length 322 at 56°C annealing temperature.

Stromal Cells, Stromal Protein

A stromal cell line was authenticated by the knock-in of β-galactosidase in the BF-2/Foxd1 locus (Hatini *et al.,* 1996) and expression of LACZ in all cells of the clone. These cells also expressed retinaldehyde dehydrogenase-2 (Raldh2; Batourina *et al.,* 2001), a second cortical stroma marker, but were negative for Pod-1 (a medullary marker; Quaggin *et al.,* 1999). Raldh2 and Pod-1 (Quaggin *et al.,* 1998) were assayed by RT-PCR at 33 cycles (Raldh2 forward: 5′-GAGGTATTATGCAGGCTGGG, reverse: 5′-GAT-GTGAGAAGCGATTGCTG; product length 322 bp at 57°C annealing temperature; Pod-1 forward: 5′-AGGAGTTTGGA-ACTTCCAACGAGA, reverse: 5′-TCTCGTACTTGTCGTTGGC-CAGGA; product length 340 at 61°C annealing temperature).

The stromal cell line was grown in DMEM, low glucose with 10% fetal calf serum. Serum-free stromal proteins were obtained by changing the culture media to DMEM (no serum) when the cells had achieved 70% confluence. The overnight culture media was then discarded and the cells were then incubated in fresh serum-free media for 2.5 days to collect stromal proteins. The stromal-conditioned medium was then concentrated on 10-kDa cut-off filters and fractionated with heparin-Sepharose and Superdex-75 gel filtration columns (Pharmacia) according to previous protocols (Barasch *et al.,* 1997). These proteins were added to the isolated cellular clusters cultured with LIF (20 ng/ml) and FGF-2/TGFα in serum-free media (Karavanova *et al.,* 1996; Barasch *et al.,* 1999b) for 7 days. Alternatively, E_{13} kidneys were cultured in MEM, 10% FCS on transwell filters with or without additional stromal proteins.

RESULTS

Identification of Cellular Clusters of Metanephric Mesenchymal Cells

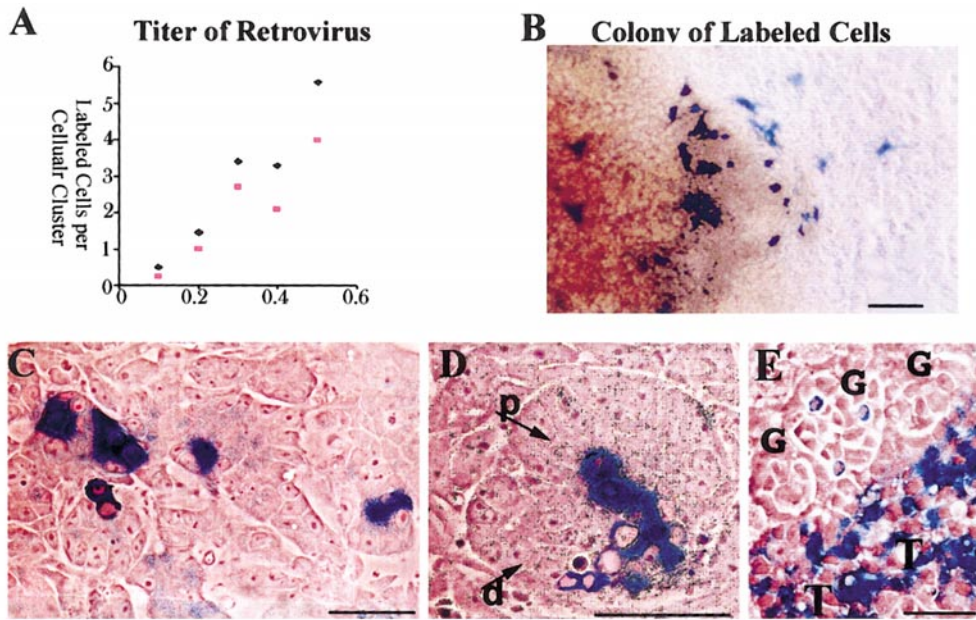
The classical view of renal development is that a uniform and naïve population of metanephric mesenchymal cells converts into epithelia in a single step after stimulation by the ureteric bud (Nordling *et al.,* 1971; Barasch *et al.,* 1999b; Plisov *et al.,* 2001). However, recent work has shown that the metanephric mesenchyme is a complex cluster of different cell types that includes epithelial progenitors at many stages of development, as well as stroma and endothelial progenitors (Hatini *et al.,* 1996; Mendelsohn *et al.,* 1999; Abrahamson *et al.,* 1998).

To visualize epithelial progenitors among the many types of metanephric mesenchymal cells, we used classical methodology to separate rat metanephric mesenchyme from its ureteric bud, and to culture the isolated metanephric mesenchyme. We previously found that metanephric mesenchyme undergoes apoptosis after isolation from its ureteric bud (Figs. 1A and 1B; Koseki *et al.,* 1992; Coles *et al.,* 1993; Barasch *et al.,* 1997), unless treated with any number of growth factors. We used a combination of FGF-2 and TGFα (FGF-2/TGFα) because these metanephric mesenchymes remain competent to form epithelia (Karavanova *et al.,* 1996; Barasch *et al.,* 1997, 1999a,b; Plisov *et al.,* 2001) and FGF-2 is secreted by the ureteric bud (Barasch *et al.,* 1997). We found that the metanephric mesenchyme contained two distinct cell populations 12–24 h after initiating cultures with FGF-2/TGFα (Figs. 1C–1F). There were refractile "cellular clusters," separated by darker, less aggregated cells. By isolating and culturing metanephric mesenchyme different stages during the initial invasion of the ureteric bud ($E_{11.5}$ to E_{14} in the rat), we found that the number of cellular clusters increased with the age of the embryo (Figs. 1C–1F). Just after the Wolffian duct arrived at the metanephric mesenchyme, but even before formation of the ureteric bud, 1.1 $\pm$ 0.3 cellular clusters ($n = 7$ at rat $E_{11.5}$) formed in the cultured, isolated metanephric mesenchyme. After entry and bifurcation of the ureteric bud in the metanephric mesenchyme, 2.1 $\pm$ 0.2 cellular clusters at $E_{12.5}$ ($n = 5$) and 3.4 $\pm$ 0.5 cellular clusters at E_{13} ($n = 6$) formed in culture. When the ureteric bud had branched into four tips ($E_{13.5}$–E_{14}), we found 4.6 $\pm$ 1.8 cellular clusters at $E_{13.5}$ ($n = 11$) and 6.2 $\pm$ 1.2 cellular clusters at rat E_{14} ($n = 15$). These data show that, when metanephric mesenchyme is maintained overnight with FGF-2/TGFα, cellular clusters form from a subset of metanephric mesenchymal cells (see below). The number of clusters is proportional to the number of tips of the ureteric bud, suggesting that the Wolffian duct and the ureteric bud stimulate the formation of this cell type.

Cellular Clusters Express Regulatory and Cellular Genes Typical of Epithelial Progenitors

To identify the cell type in the cellular clusters, we analyzed a series of genes and proteins that are differentially expressed in the developing kidney. Transcription factors WT-1 (Kreidberg *et al.,* 1993) and Pax-2 (Rothenpieler and Dressler, 1993) and the secreted factor Wnt-4 (Vainio and Muller, 1997; Stark *et al.,* 1994) are most highly expressed by cells surrounding tips of the ureteric bud *in vivo* (Figs. 2A, 2C, and 2E). This region excludes BF-2/Foxd1 and tenascin that identify renal stroma, which instead appear in a peripheral compartment of the developing kidney (Figs. 2G and 2I; Hatini *et al.,* 1996). The cellular clusters formed *in vitro* were similar to cells near the ureteric bud since they intensely expressed WT-1 (Fig. 2B), Pax-2 (Fig. 2D), and Wnt-4 (Fig. 2F), but excluded BF-2/Foxd1 and tenascin, which instead appear at the periphery of the cellular clusters (Figs. 2H and 2J). Hence, it is likely that the cellular

© 2002 Elsevier Science (USA). All rights reserved.



A
LIF - + - +
97
66
46
30
21
phospho-STAT3 STAT3

B
Cellular Clusters
No RNA
SOCS1 SOCS2 SOCS3 CIS
SOCS2 SOCS3 CIS
LIF - + - + - + - +
RT + + + + + + + + + + +
G3PDH
G3PDH

C
D
FGF-2/TGF α
FGF-2/TGFα/LIF

5 E FGF-2/TGF α
F FGF-2/TGFα/LIF
G FGF-2/TGFα/LIF

FIG. 4. Lineage tagging of the cellular clusters with a replication-defective retrovirus demonstrates multipotent epithelial progenitors. (A) Cellular clusters were extracted from the metanephric mesenchyme and exposed to a serial dilution of retrovirus 0–0.5 ml of virus/ml of culture. Forty-eight hours later, the cellular clusters were processed for β-galactosidase histochemistry. Note the linear dose-response of viral supernatant and number of infected cells. Two independent titrations are shown. (B–E) Cellular clusters were exposed to a clonal dilution of retrovirus and then cultured for 7 days with FGF-2/TGFα and LIF. (B) The edge of one cellular cluster contains a colony of infected daughter cells. The infected cells appear dark blue. Note the compact association of the infected cells. No other tagged cells were present in the cellular cluster. (C–E) Serial sections of three different infected cellular clusters show tagged epithelia. (C) The infected cells

© 2002 Elsevier Science (USA). All rights reserved.

clusters derive from mesenchymal cells near the ureteric bud. Additionally, since Pax-2, WT-1, and Wnt-4 have cell-autonomous functions during cell conversion, the data suggest that the cellular clusters are epithelial progenitors.

To determine whether the cellular clusters also had epithelial characteristics, we examined their morphology and a series of adhesion molecules and matrix proteins that are differentially expressed during cell conversion. The cells in the clusters were less elongated than other metanephric mesenchymal cells (Fig. 3A). However, there were no obvious basement membranes, or lateral or apical specializations to suggest that these cells were polarized epithelia (not shown). In fact, while the earliest epithelial collagen, collagen IV (Ekblom *et al.,* 1981), was present (Fig. 3B), other basement membrane proteins that are known to appear later in cell conversion, including laminin $\alpha5$ (Fig. 3C; Ekblom *et al.,* 1981; Miner *et al.,* 1997), were absent. Likewise, intercellular adhesion molecules that characterize mesenchymal cells and the earliest stages of conversion, including NCAM (Fig. 3D; Klein *et al.,* 1988) and cadherin-6 (Fig. 3E; Mah *et al.,* 2000; Cho *et al.,* 1998), were expressed, but proteins that appear later in cell conversion, such as ZO-1 (tight junctions; Mah *et al.,* 2000; Cho *et al.,* 1998) and E-cadherin (adherens junctions; Vestweber *et al.,* 1985), were not detectable (Fig. 3E). These data show that the cellular clusters are mesenchymal cells at an initial stage of epithelial conversion.

Cellular Clusters Convert to Epithelia

To determine the fate of the cellular clusters, we used an *in vitro* model of induction. As we previously found, culture with FGF-2/TGFα for as long as 2 weeks ($n = 24$) did not produce epithelia (Barasch *et al.,* 1997). However, when the ureteric bud factor LIF (Barasch *et al.,* 1999b; Plisov *et al.,* 2001) was added together with FGF-2/TGFα, there was rapid cell conversion. Within 2 days of adding LIF (20 ng/ml), the cellular clusters expressed epithelial adhesion proteins E-cadherin and ZO-1 (Fig. 3E) and the epithelial matrix protein laminin α_5 (not shown). By 4 days, the cells reorganized from a haphazard array (Fig. 3A) into small chains (Fig. 3F) that were mounted on islands of collagen IV$^+$, laminin $\alpha5^+$ matrix (Figs. 3G and 3H). Cells in these chains had characteristic features of epithelial polarity, including a basement membrane, a basally located nucleus, a supranuclear golgi, and specializations typical of the apical membrane (Fig. 3I). By 7 days, the cellular clusters were filled with fully polarized tubules (Fig. 3J) bounded by continuous basement membranes (Figs. 3K and 3L). These data show that, while the cellular clusters do not spontaneously produce epithelia, they are competent epithelial progenitors.

Cellular Clusters Contain Multipotent Epithelial Progenitors

Previous work showed that the metanephric mesenchyme contains multipotent epithelial progenitors (Herzlinger *et al.,* 1992) that give rise to different types of epithelia. The existence of this cell was suggested by analysis of the daughters of a single cell that had been tagged with a replication defective retrovirus (Cepko *et al.,* 2000). Because the analysis was retrospective, the cellular characteristics of the progenitor could not be identified. To determine whether the cellular clusters are multipotent cells, we isolated these cells (see Methods) and labeled them with a clonal titer of a replication defective retrovirus. The clonal titer was determined by infecting isolated cellular clusters ($n = 140$) with a serial dilution of virus and then counting labeled cells 48 h later after culture with FGF-2/TGFα and LIF (Fig. 4A). For our studies, we chose a titer that labeled only 25% of the explants (average 0.2 ± 0.1 SEM labeled cells/cell cluster; $n = 20$), which is below the single hit concentration of virus predicted by a Poisson distribution (Herzlinger *et al.,* 1992). Additional evidence supporting clonal labeling included the presence of only one colony/cellular cluster and a compact association of labeled cells even after 1 week of culture (Fig. 4B).

---

in this cluster were found in neighboring tubules. No other infected cells were present in the cellular cluster. (D) This colony only has tagged cells across the C-shaped nephron. P, proximal; D, distal domains. (E) Tagged cells are found in two adjacent tubules (T) and in one of three glomeruli (G) in podocytes. Bars, (B) 150 μm; (C, E) 20 μm; (D) 30 μm.

FIG. 5. Cellular clusters of epithelial progenitors respond to LIF. (A) Freshly isolated cellular clusters were treated with LIF for 10 min and the phosphorylation of STAT-3 at tyrosine 705 was detected with specific antibodies. The same blot was then reprobed with an antibody that recognizes all species of STAT-3, showing equal loading of the gel. (B) Freshly isolated cellular clusters were treated with LIF for 30 min and then assayed for the expression of phospho-STAT-3-activated genes called "suppressors of cytokine signaling" (SOCS) and the similar cytokine-inducible SH2-containing protein (CIS). The upregulated expression of SOCS2 and CIS genes after treatment with LIF indicates robust and productive signaling in the isolated cellular clusters. [RT-PCR: with reverse transcriptase (+); "No RNA" is the complete RT-PCR without template; G3PDH is a control amplification]. (C, D) Isolated cellular clusters were cultured for 12 h with FGF-2/TGFα or with FGF-2/TGFα + LIF and were then pulsed with BrdU. Culture with LIF increased the incorporation of BrdU, as detected by nuclear staining using antibodies for BrdU. Nuclear counterstain with Sytox, not shown. (E–G) Isolated cellular clusters cultured with either FGF-2/TGFα or FGF-2/TGFα + LIF for 7 days. (E) Culture with FGF-2/TGFα allows survival of the cellular cluster but limited cell proliferation. (F) In contrast, culture with FGF-2/TGFα + LIF induces massive proliferation. (G) Isolated cellular clusters treated with FGF-2/TGFα + LIF were stained with anti-tenascin to detect contaminating stromal cells. Only 10 stromal cells are found at the edge of the cellular cluster. Bars, (C–G) 50 μm.

© 2002 Elsevier Science (USA). All rights reserved.

When isolated cellular clusters ($n = 345$) were clonally labeled and then recultured for 7 days with LIF and FGF-2/TGFα, 84 cellular clusters had labeled colonies composed of 9.9 ± 1.6 SEM-labeled cells (range 4–28). In 36, virally labeled cells were present in neighboring tubules (Fig. 4C), and in 17 colonies, we found labeling in different parts of the developing C-shaped body (Fig. 4D), including presumptive distal and proximal tubules. In 6 colonies both glomeruli and tubules had labeled cells (Fig. 4E). In many colonies ($n = 65$), there were labeled, spindle-shaped cells that might be undifferentiated mesenchyme. These findings are characteristic of multipotent progenitors (Herzlinger *et al.,* 1992). The data show that determination of epithelial type occurs after the expression of Pax-2, WT-1, and Wnt-4 and after the initial steps of cellular reorganization that accompany epithelial conversion.

Ureteric Bud Factor Converts Isolated Cellular Clusters into Epithelia: No Requirement for Renal Stroma

Because the cellular clusters could respond to LIF and convert into epithelia (87% of the isolated cellular clusters; $n > 600$) after isolation from the metanephric mesenchyme, it appears that LIF directly regulates the fate of epithelial progenitors. In fact, the LIF receptor was specifically expressed by these cells (not shown; Plisov *et al.,* 2001) and they specifically bound fluorescent LIF (not shown). In addition, LIF activated second messengers (pSTAT-3; Fig. 5A) and downstream genes (SOCS, STAT-activated "suppressors of cytokine signaling"; Alexander *et al.,* 1999; Naka *et al.,* 1999; Fig. 5B) equally well in the isolated cellular clusters, as in the metanephric mesenchyme; nor was the proliferative response to LIF changed by the isolation. When isolated cellular clusters were cultured overnight with FGF-2/TGFα and then labeled with BrdU (Fig. 5C), 20% of the total nuclei (visualized with Sytox) were labeled, whereas when LIF was included with FGF-2/TGFα, 100% of the nuclei were labeled (Fig. 5D). Also similar to cultures of metanephric mesenchyme, the isolated cellular clusters failed to grow over 7 days (Fig. 5E) with FGF-2/TGFα, but in the presence of LIF, there was massive growth (Fig. 5F).

To determine whether stroma and endothelial cells were present and contributing to the response of the isolated cellular clusters to LIF, we examined for tenascin and BF-2/Foxd1 (stroma) and for von Willebrand, (endothelia), but found none. After 7 days of culture, the isolated cellular clusters had at most 20 tenascin$^+$ stromal cells/10^4 cells (Fig. 5G; $n = 32$) at their periphery and there were no von Willebrand$^+$ endothelia. We conclude that the ureteric bud controls proliferation and conversion of Pax-2, WT-1, and Wnt-4 progenitors, and that if stroma did contribute to epithelial conversion, its contribution could be readily bypassed in culture by adding molecules like LIF, that are specifically expressed by the ureteric bud with little, if any, input from renal stroma or endothelia. Hence, there is no absolute requirement for stroma or endothelia during the induction of epithelia by ureteric bud LIF.

Stroma Modulate Cell Conversion, Tubulogenesis, and Glomerulogenesis

While the initial responses of epithelial progenitors to LIF were not altered by separation from the stroma, we were surprised by a change in the type of epithelia induced by LIF. When intact metanephric mesenchyme was cultured with FGF-2/TGFα and LIF, it produced 5 ± 0.9 glomeruli and 127 ± 16 E-cadherin$^+$ tubules per metanephric mesenchyme ($n = 29$). In contrast, a single isolated cellular cluster produced 36 ± 5 SEM ($n = 42$) fully formed "capillary loop" phase glomeruli and 58 ± 11 tubules. Glomeruli were readily identified by the classical morphology of condensed visceral podocytes, urinary space, and a Bowman's capsule (Figs. 6A and 6B). Expression of peanut lectin-binding sites (Fig. 6C; Laitinen *et al.,* 1987) and podocalyxin (Fig. 6D; Kerjaschki *et al.,* 1984) confirmed the formation of podocytes. The tubules expressed E-cadherin, a marker of the distal tubule (Fig. 6E; Cho *et al.,* 1998).

The enhanced glomerulogenesis in the isolated progenitors could have resulted from the removal of a factor during the extraction of the cellular clusters. Because BF-2$^+$/Foxd1 stromal cells were depleted by the isolation procedure, we tested whether stroma produced this factor by incubating the isolated cellular clusters with media conditioned by a BF-2$^+$/Foxd1 renal stromal cell line (Fig. 7A). Unlike proteins secreted from ureteric bud cell lines (LIF and other proteins), conditioned media from the stromal cells never had any growth or inductive activity. However, stromal proteins (50 μg/ml) changed the type of epithelia that was induced by LIF. Only short tubules (Figs. 7B and 7C) that expressed E-cadherin (47 ± 6 E-cadherin$^+$ tubules/epithelial precursor, $n = 38$; Fig. 7F) could be induced by LIF + FGF-2/TGFα when stromal proteins were added. The epithelia, in fact, were expanded into sheets of cells (Fig. 7F). In contrast, we did not find any glomeruli in serial sections, nor were peanut lectin-binding sites (Fig. 7D) or podocalyxin (Fig. 7E) expressed.

We quantified the response of epithelial precursors to stroma and ureteric bud factors by assaying podocalyxin and E-cadherin by immunoblot. We found that the isolation of the cellular clusters from metanephric mesenchyme increased the yield of podocalyxin/mg protein, and conversely, the addition of stromal proteins abolished its expression (Fig. 8A). In contrast, stromal factors enhanced the expression of E-cadherin/mg protein 10-fold, compared to induction in the isolated cellular clusters in the absence of stromal proteins (Fig. 8B). These data confirm that separation of the cellular clusters from stroma enhances glomerulogenesis, while replacement of the stroma abolishes glomerulogenesis.

© 2002 Elsevier Science (USA). All rights reserved.

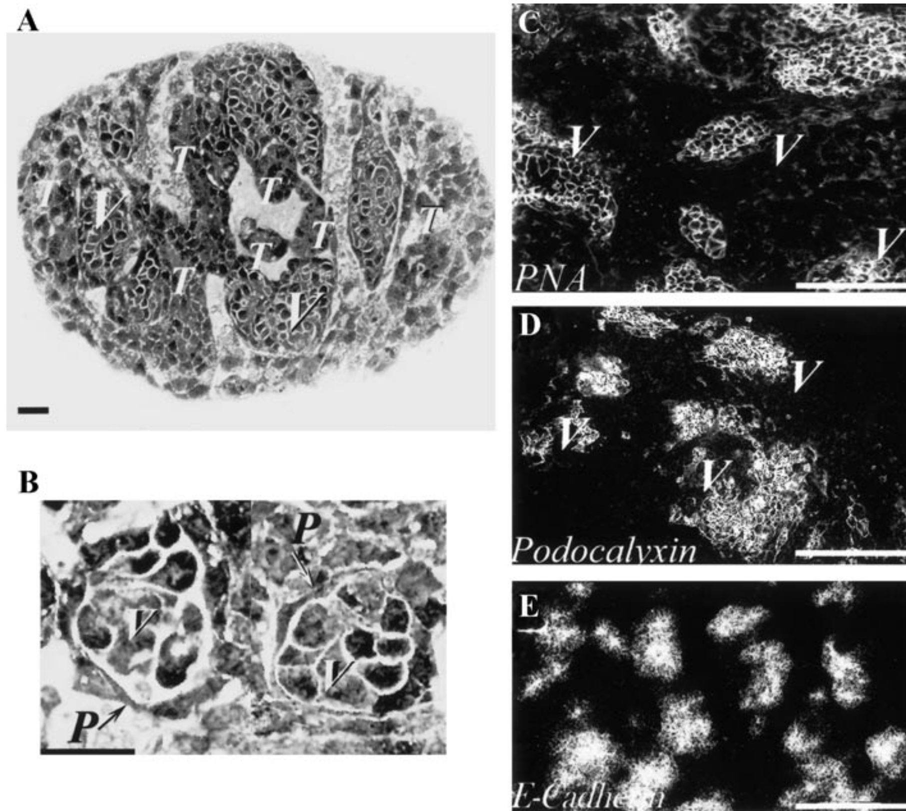


FIG. 6. Extensive glomerulo- and tubulogenesis in isolated cellular clusters treated with FGF-2/TGFα + LIF for 7 days. (A, B) Many more glomeruli (sevenfold) form in the isolated cellular clusters compared with similarly cultured metanephric mesenchyme. Glomeruli were identified by their centrally located visceral podocytes (*V*), a urinary space, and parietal cells (*P*) of Bowman's Capsule. T, tubules. (C) In addition, the classical markers of podocytes, peanut lectin-binding sites and (D) podocalyxin were expressed. (E) Extensive tubulogenesis is indicated by the expression of E-cadherin. Bars: (A, B) 15 μm; (C–E) 200 μm.

To test whether the addition of excess stromal proteins could modify the development of epithelia in intact kidneys, we cultured E_{13} rat kidneys with aliquots of media conditioned by BF-2^+/Foxd1 stromal cells (50 μg/ml) and counted the number of glomeruli. Glomeruli were detected by staining for both podocalyxin and WT-1 (which is highly expressed by podocytes once nephrons form). We found that the controls had 23 $\pm$ 7 podocalyxin$^+$ glomeruli/kidney ($n = 30$), while the paired kidneys treated with the stromal-conditioned media had only 5 $\pm$ 2 podocalyxin$^+$ glomeruli (Fig. 9; n = 30). However, just like the isolated cellular clusters, E-cadherin$^+$ tubular epithelia were still present. These data indicate that, while BF-2^+/Foxd1 cells do not stimulate the growth or epithelialization of epithelial progenitors, they produce factors that modulate epithelial type. Preliminary fractionation of the stromal cell-conditioned media revealed a heparin binding (trypsin-sensitive) protein (eluting at 0.2 M NaCl) with a molecular weight of 55 kDa, and p*I* 7.0 that permitted the formation of E-cadherin$^+$ epithelia without glomeruli.

DISCUSSION

The cardinal feature of renal development is the formation of many types of epithelia from mesenchymal cells. However, basic information about this cellular conversion has been lacking. It is necessary to isolate the epithelial progenitor, to identify the stages of its maturation, and to find regulators of its progression.

In the current paper, we have identified and isolated clusters of epithelial progenitors from the metanephric mesenchyme. These are the earliest epithelial progenitors that have been identified, authenticated by functional assay, and isolated. The isolated progenitors are useful for two reasons. In kidney, the development of epithelia is the result of a series of reciprocating signaling pathways between many different cell types. These interactions include bidirectional signaling between ureteric bud and epithelial progenitors (Grobstein, 1955; Schuchardt *et al.,* 1994; Barasch *et al.,* 1999a), between ureteric bud and renal stroma (Batourina *et al.,* 2001), and between epithelial

© 2002 Elsevier Science (USA). All rights reserved.

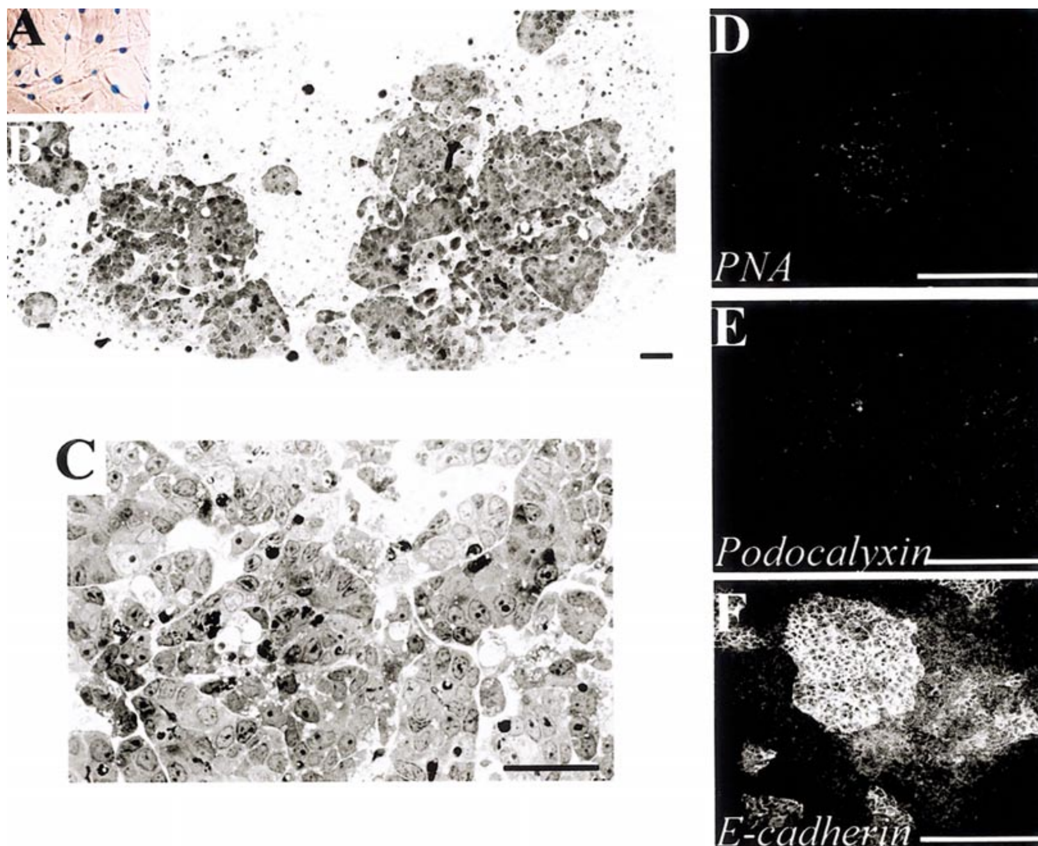


FIG. 7. Extensive tubulogenesis but no glomerulogenesis in isolated cellular clusters treated with FGF-2/TGFα + LIF and a conditioned media prepared from the BF-2/Foxd1$^+$ renal stromal cells for 7 days. (A) The BF-2/Foxd1 cell line was authenticated by the knock-in of LACZ in the BF-2 locus and expression of β-galactosidase every cell of the clone. (B, C) When stromal proteins (50 μg/ml) were included, extensive epithelialization and tubulogenesis was found without a single glomerulus. (D) There was no expression of podocyte markers, peanut lectin binding, or (E) podocalyxin. (F) Epithelialization and tubulogenesis was indicated by the expression of E-cadherin. Note the expanded domains of E-cadherin. Bars, (B) 15 μm; (C–E) 200 μm.

progenitors and renal stroma (Hatini *et al.,* 1996; Dudley *et al.,* 1999). Using isolated epithelial progenitors, we have assayed the effect of inducing molecules on the development of epithelia without the confounding variable of reciprocating signaling. Our approach is similar to the one used by Grobstein (1955), who defined the interaction of the ureteric bud and metanephric mesenchyme by separating these cell types.

Isolated epithelial progenitors also provide a single population of cells in which to simultaneously test the relationship between transcriptional regulators (e.g., Pax-2, WT-1; Dressler, 1995; Kreidberg *et al.,* 1993), cellular proteins (e.g., E-cadherin; Ekblom *et al.,* 1981, 1990, 1994; Ekblom, 1989; Cho *et al.,* 1998; Mah *et al.,* 2000; Vainio *et al.,* 1992), signaling molecules (e.g., Wnt-4; Vainio and Muller, 1997; Stark *et al.,* 1994; Plisov *et al.,* 2001), and developmental potential (Herzlinger *et al.,* 1992). In contrast, previous works from different laboratories are often difficult to compare. For example, it is not known whether a cell that expresses Wnt-4 or E-cadherin is a multipotent progenitor, because cells with those cellular characteristics had not been specifically assayed by lineage tagging. Further, the cellular characteristics of multipotent progenitors were not known since they were defined by a retrospective analysis (Herzlinger *et al.,* 1992). In contrast, by isolating cellular clusters of epithelial progenitors, we have examined cellular proteins, transcription factors, and the developmental potential of a single subset of cells at a specific point during cellular conversion.

Stages of Mesenchymal-to-Epithelial Conversion

The cellular clusters could be distinguished from other mesenchymal cells by their intense expression of Pax-2, WT-1, and Wnt-4, by a distinct organization of the cells, and by expression of collagen IV and cadherin-6, which are early epithelial proteins. These data indicate that these cells have already progressed in the pathway that leads to epithelial-

© 2002 Elsevier Science (USA). All rights reserved.

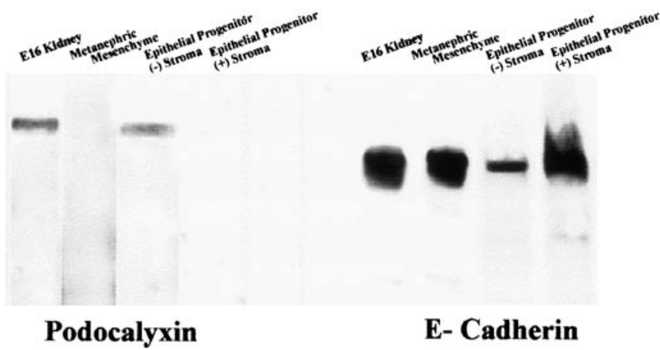


FIG. 8. Stroma modifies epithelial cell type. Extraction of epithelial progenitors from the metanephric mesenchyme enhanced expression of the glomerular marker podocalyxin, but addition of media conditioned by BF-2/Foxd1^{+} stromal cells abolished expression. Conversely, E-cadherin expression was enhanced to the level found in the metanephric mesenchyme when the isolated epithelial progenitors were treated with stromal proteins. Cultures with LIF and FGF-2/TGFα, with and without additional stromal proteins (50 μg/ml), as indicated.

ization. However, the cells failed to express markers of later stages of development, such as E-cadherin, ZO-1, or laminin α5, or to develop evidence of apical–basal polarity, despite survival for many days in culture in the presence of FGF-2/TGFα. Hence, the initial stages of mesenchymal-to-epithelial conversion can be readily dissociated from the completion of epithelialization. These findings are reminiscent of a number of observations made *in vivo* in the kidney. Pax-2 and WT-1 are expressed many days before overt epithelial development in the kidney (Donovan *et al.*, 1999; Obara-Ishihara *et al.*, 1999) and WT-1^{+}, Pax-2^{+}, and Wnt-4^{+} cells accumulate without epithelialization in BF-2/Foxd1$^{-/-}$ and Pod-1$^{-/-}$ animals (Hatini *et al.*, 1996; Quaggin *et al.*, 1999), indicating that additional stimuli must activate WT-1^{+}, Pax-2^{+}, and Wnt-4^{+} cells. Likewise, in other developing systems, essential regulators like Pax genes are expressed much before the mature phenotype appears and their expression can be dissociated from completion of the program. For example, in the developing inner ear (Groves and Bronner-Fraser, 2000), olfactory placode (Walther and Gruss, 1991), retina (Marquardt *et al.*, 2001), and trigeminal placode (Stark *et al.*, 1997), Pax genes are expressed much before overt morphogenesis, indicating that both early and late stimuli must be active. The development of peripheral neurons also proceeds by a series of inductions (Groves *et al.*, 1995).

It is likely that both the Wolffian duct and the ureteric bud produce molecules that regulate different stages of induction. The Wolffian duct is likely to induce the initial stage of epithelial transition because Pax-2 and WT-1 (Donovan *et al.*, 1999; Obara-Ishihara *et al.*, 1999) are expressed by cells adjacent to the growing duct before the appearance of the ureteric bud. This inducing factor is not known, however. LIF and other IL-6 cytokines, FGFs, and EGF family members are not candidates for this activity because early metanephric mesenchyme (rat $E_{11.5}$), which contain only one cellular cluster, failed to develop additional WT-1^{+}, Pax-2^{+}, and Wnt-4^{+} cells after treatment with these factors (not shown). In contrast, the WT-1^{+}, Pax-2^{+}, and Wnt-4^{+} cells are rapidly converted to epithelia after addition of LIF or any of the other IL-6 cytokines that are expressed by the ureteric bud (Hara *et al.*, 1998; Sheng *et al.*, 1996; Barasch *et al.*, 1999b; Plisov *et al.*, 2001). WT-1^{+}, Pax-2^{+}, and Wnt-4^{+} cells specifically express the LIF receptor (Plisov *et al.*, 2001) and LIF binding sites (not shown) and

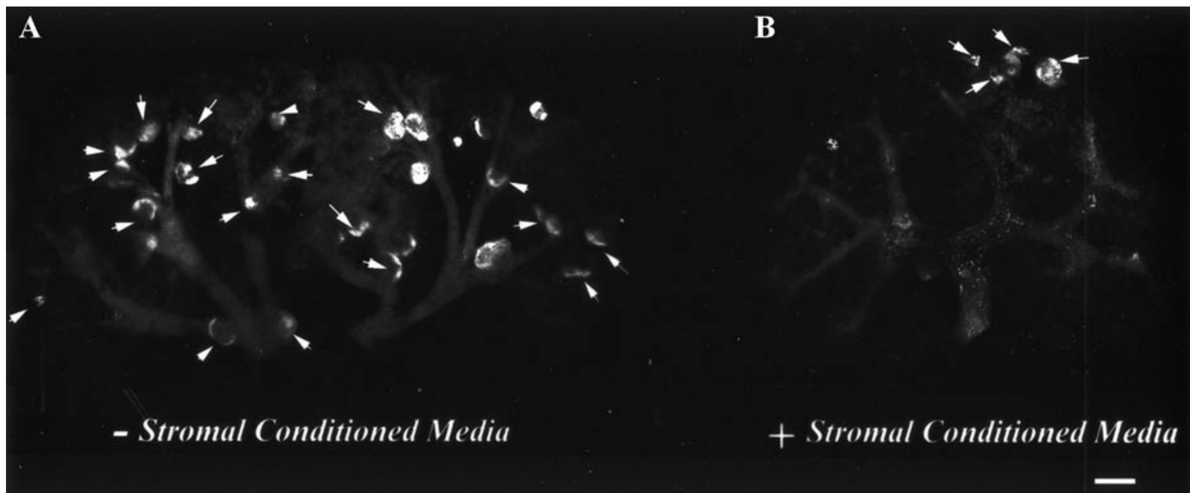


FIG. 9. (A) Embryonic kidneys produce many podocalyxin^{+} glomeruli (arrows) over 5 days in culture. (B) However, the addition of media conditioned by BF-2/Foxd1^{+} stromal cells (50 μg/ml) nearly abolished glomerulogenesis. Bar, 75 μm.

© 2002 Elsevier Science (USA). All rights reserved.

activate STAT-3 (Barasch *et al.,* 1999b), demonstrating that they are targeted by stimuli that induce conversion. In fact, LIF's action is reminiscent of a late stage in epithelial stem cell maturation, where limited proliferation (3–5 cycles of "transit amplifying cells"; Booth and Potten, 2000; Lehrer *et al.,* 1998; Jones and Watt, 1993; Potten and Morris, 1988) and expression of the mature phenotype occur in tandem. Similarly, LIF targets single Pax-2$^+$, WT-1$^+$, and Wnt-4$^+$ cells to undergo two to five cell cycles to produce 9.9 ± 1.6 SEM (range 4–28) mature, fully polarized epithelia. Hence, we propose that similar to other inductions, successive signals regulate development of the mature epithelial phenotype.

Regulation of Epithelial Cell Type

The mechanisms that govern the generation of different epithelial cell types from induced metanephric mesenchyme have not been explored. For example, it is not known when an epithelial progenitor (or an epithelial cell) commits to form a glomerular or a tubular epithelial cell. We found that Pax-2$^+$, WT-1$^+$, and Wnt-4$^+$ cells have a multipotent phenotype as previously described (Herzlinger *et al.,* 1992); hence, the choice of cell type occurs after expression of these molecules, during completion of epithelialization. LIF does not bias the choice of epithelia, but the formation of glomeruli is negatively regulated by stroma. The evidence that stroma regulates epithelial type includes the induction of over 100 E-cadherin$^+$ tubules but few glomeruli in metanephric mesenchyme, where BF-2/Foxd1$^+$ stromal cells surround epithelial progenitors and conversely the formation of many more glomeruli when the epithelial progenitors are depleted of stromal cells. In fact, the readdition of stromal proteins inhibited glomerulogenesis. These data indicate that the stroma is a regulator of epithelial type in the development of the nephron. Stroma could directly inhibit development of certain types of epithelia, but remain permissive for other types. Alternatively, the choice of epithelial type may be more akin to the formation of different hemopoietic cells, where "lineage-restricted growth factors" have been found to select among developmental programs that are active in a multipotent progenitor (Cross *et al.,* 1994; Wu *et al.,* 1995; Socolovsky *et al.,* 1998). Similarly, the coexpression of Pax-2 and WT-1 in our isolated (multipotent) cells might predict that they could form either tubular or glomerular epithelia, because these factors later localize to, and exclusively regulate, tubular or glomerular epithelia, respectively (e.g., Dressler, 1995; Majumdar *et al.,* 2000); hence, perhaps stroma biases renal induction by modulating Pax-2 and/or WT-1 with a "lineage restricted growth factor." The stromal activity selects and expands one cell type from among the developmental potentials found in the Pax-2$^+$, WT-1$^+$ progenitors. Consistent with this model for the function of renal stroma is a wealth of data showing that stroma patterns epithelia in many organs. For example, different lobes of the prostate (Hayward *et al.,* 1998; Cunha, 1994), segments of the female reproductive tract (Miller and Sassoon, 1998), and regions of the airway undergoing branching morphogenesis (Spooner and Wessells, 1970) contain different epithelia as a result of signaling by stroma. Even adult cells may be regulated by stroma (Neubauer *et al.,* 1983; Cunha *et al.,* 1985), including renal epithelia, which are thought to be respecified by interstitial renal diseases (Marcussen, 1995; Okada *et al.,* 2000).

CONCLUSION

By identifying and isolating an epithelial progenitor from metanephric mesenchyme, we have shown that two sets of signals regulate the production of epithelia. Signaling from the ureteric bud induces a wide variety of epithelia to form, but signaling from stroma, in combination with ureteric bud factors, produces only one class of epithelia. These effects are shown in epithelial progenitors at a specific stage of their development, when they express essential regulators of epithelial conversion, but have yet to form polarized epithelia.

ACKNOWLEDGMENTS

We thank G. Dressler, M. Farquhar, A. McMahon, J. Miner, Larry Holtzman, and David Salant for probes and advice. We thank Qais Al-Awqati and the referees for critical review. J.B. is an Established Investigator of the American Heart and is supported by a Research Grant (FY00165) from the March of Dimes Birth Defects Foundation and by the NIH (DK 55388).

REFERENCES

Abrahamson, D. R., Robert, B., Hyink, D. P., St John, P. L., and Daniel, T. O. (1998). Origins and formation of microvasculature in the developing kidney. *Kidney Int. Suppl.* **67,** S7–S11.

Alexander, W. S., Starr, R., Metcalf, D., Nicholson, S. E., Farley, A., Elefanty, A. G., Brysha, M., Kile, B. T., Richardson, R., Baca, M., Zhang, J. G., Willson, T. A., Viney, E. M., Sprigg, N. S., Rakar, S., Corbin, J., Mifsud, S., DiRago, L., Cary, D., Nicola, N. A., and Hilton, D. J. (1999). Suppressors of cytokine signaling (SOCS): Negative regulators of signal transduction. *J. Leukoc. Biol.* **66,** 588–592.

Barasch, J., Pressler, L., Connor, J., and Malik, A. (1996). A ureteric bud cell line induces nephrogenesis in two steps by two distinct signals. *Am. J. Physiol.* **271,** F50–F61.

Barasch, J., Qiao, J., McWilliams, G., Chen, D., Oliver, J. A., and Herzlinger, D. (1997). Ureteric bud cells secrete multiple factors, including bFGF, which rescue renal progenitors from apoptosis. *Am. J. Physiol.* **273,** F757–F767.

Barasch, J., Yang, J., Qiao, J., Tempst, P., Erdjument-Bromage, H., Leung, W., and Oliver, J. A. (1999a). Tissue inhibitor of metalloproteinase-2 stimulates mesenchymal growth and regulates epithelial branching during morphogenesis of the rat metanephros. *J. Clin. Invest.* **103,** 1299–1307.

Barasch, J., Yang, J., Ware, C. B., Taga, T., Yoshida, K., Erdjument-Bromage, H., Tempst, P., Parravicini, E., Malach, S., Aranoff, T.,

© 2002 Elsevier Science (USA). All rights reserved.

and Oliver, J. (1999b). Mesenchymal to epithelial conversion in rat metanephros is induced by LIF. *Cell* **99,** 377–386.

Barasch, J. (2001). Genes and proteins involved in mesenchymal to epithelial transition. *Curr. Opin. Nephrol. Hypertens.* **10,** 429–436.

Batourina, E., Gim, S., Bello, N., Shy, M., Clagett-Dame, M., Srinivas, S., Costantini, F., and Mendelsohn, C. (2001). Vitamin A controls epithelial/mesenchymal interactions through Ret expression. *Nat. Genet.* **27,** 74–78.

Booth, C., and Potten, C. S. (2000). Gut instincts: Thoughts on intestinal epithelial stem cells. *J. Clin. Invest.* **105,** 1493–1499.

Cepko, C. L., Ryder, E., Austin, C., Golden, J., Fields-Berry, S., and Lin, J. (2000). Lineage analysis with retroviral vectors. *Methods Enzymol.* **327,** 118–145.

Coles, H. S., Burne, J. F., and Raff, M. C. (1993). Large-scale normal cell death in the developing rat kidney and its reduction by epidermal growth factor. *Development* **118,** 777–784.

Cho, E. A., Patterson, L. T., Brookhiser, W. T., Mah, S., Kintner, C., and Dressler, G. R. (1998). Differential expression and function of cadherin-6 during renal epithelium development. *Development* **125,** 803–812.

Cross, M. A., Heyworth, C. M., Murrell, A. M., Bockamp, E. O., Dexter, T. M., and Green, A. R. (1994). Expression of lineage restricted transcription factors precedes lineage specific differentiation in a multipotent haemopoietic progenitor cell line. *Oncogene* **9,** 3013–3016.

Cunha, G. R., Bigsby, R. M., Cooke, P. S., and Sugimura, Y. (1985). Stromal–epithelial interactions in adult organs. *Cell Differ.* **17,** 137–148.

Cunha, G. R. (1994). Role of mesenchymal–epithelial interactions in normal and abnormal development of the mammary gland and prostate. *Cancer* **74**(Suppl.), 1030–1044.

Donovan, M. J., Natoli, T. A., Sainio, K., Amstutz, A., Jaenisch, R., Sariola, H., and Kreidberg, J. A. (1999). Initial differentiation of the metanephric mesenchyme is independent of WT1 and the ureteric bud. *Dev. Genet.* **24,** 252–262.

Dressler, G. R., and Douglass, E. C. (1992). Pax-2 is a DNA-binding protein expressed in embryonic kidney and Wilms tumor. *Proc. Natl. Acad. Sci. USA* **89,** 1179–1183.

Dressler, G. R. (1995). Transcription factors in renal development: The WT1 and Pax-2 story. *Semin. Nephrol.* **15,** 263–271.

Dudley, A. T., Godin, R. E., and Robertson, E. J. (1999). Interaction between FGF and BMP signaling pathways regulates development of metanephric mesenchyme. *Genes Dev.* **13,** 1601–1613.

Ekblom, P., Lehtonen, E., Saxen, L., and Timpl, R. (1981). Shift in collagen type as an early response to induction of the metanephric mesenchyme. *J. Cell Biol.* **89,** 276–283.

Ekblom, P. (1989). Developmentally regulated conversion of mesenchyme to epithelium. *FASEB J.* **3,** 2141–2150.

Ekblom, M., Klein, G., Mugrauer, G., Fecker, L., Deutzmann, R., Timpl, R., and Ekblom, P. (1990). Transiently and locally restricted expression of laminin A chain mRNA by developing epithelial cells during kidney organogenesis. *Cell* **60,** 337–346.

Ekblom, P., Ekblom, M., Fecker, L., Klein, G., Zhang, H. Y., Kadoya, Y., Chu, M. L., Mayer, U., and Timpl, R. (1994). Role of mesenchymal nidogen for epithelial morphogenesis in vitro. *Development.* **120,** 2003–2014.

Grobstein, C. (1955). Inductive interaction in the development of the mouse metanephros. *J. Exp. Zool.* **130,** 319–339.

Groves, A. K., George, K. M., Tissier-Seta, J. P., Engel, J. D., Brunet, J. F., and Anderson, D. J. (1995). Differential regulation of transcription factor gene expression and phenotypic markers in developing sympathetic neurons. *Development* **121,** 887–901.

Groves, A. K., and Bronner-Fraser, M. (2000). Competence, specification and commitment in otic placode induction. *Development* **127,** 3489–3499.

Hara, T., Tamura, K., de Miguel, M. P., Mukouyama, Y., Kim, H., Kogo, H., Donovan, P. J., and Miyajima, A. (1998). Distinct roles of oncostatin M and leukemia inhibitory factor in the development of primordial germ cells and sertoli cells in mice. *Dev. Biol.* **201,** 144–153.

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.

Hayward, S. W., Haughney, P. C., Rosen, M. A., Greulich, K. M., Weier, H. U., Dahiya, R., and Cunha, G. R. (1998). Interactions between adult human prostatic epithelium and rat urogenital sinus mesenchyme in a tissue recombination model. *Differentiation* **63,** 131–140.

Herzlinger, D., Koseki, C., Mikawa, T., and al-Awqati, Q. (1992). Metanephric mesenchyme contains multipotent stem cells whose fate is restricted after induction. *Development* **114,** 565–572.

Hirano, T., Ishihara, K., and Hibi, M. (2000). Roles of STAT3. in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors. *Oncogene* **19,** 2548–2556.

Horb, M. E., and Slack, J. M. (2001). Endoderm specification and differentiation in Xenopus embryos. *Dev. Biol.* **236,** 330–343.

Jones, P. H., and Watt, F. M.(1993). Separation of human epidermal stem cells from transit amplifying cells on the basis of differences in integrin function and expression. *Cell* **73,** 713–724.

Karavanova, I., Dove, L., Resau, J., and Perantoni, A. (1996). Conditioned media from a rat ureteric bud cell line in combination with bFGF induces complete differentiation of isolated metanephric mesenchyme. *Development* **122,** 4159–4167.

Kerjaschki, D., Sharkey, D. J., and Farquhar, M. G. (1984). Identification and characterization of podocalyxin: The major sialoprotein of the renal glomerular epithelial cell. *J. Cell Biol.* **98,** 1591–1596.

Klein, G., Langegger, M., Goridis, C., and Ekblom, P. (1988). Neural cell adhesion molecules during embryonic induction and development of the kidney. *Development* **102,** 749–761.

Koseki, C., Herzlinger, D., and al-Awqati, Q. (1992). Apoptosis in metanephric development. *J. Cell Biol.* **119,** 1327–1333.

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.

Laitinen, L., Virtanen, I., and Saxen, L. (1987). Changes in the glycosylation pattern during embryonic development of mouse kidney as revealed with lectin conjugates. *J. Histochem. Cytochem.* **35,** 55–65.

Lehrer, M. S., Sun, T. T., and Lavker, R. M. (1998). Strategies of epithelial repair: Modulation of stem cell and transit amplifying cell proliferation. *J. Cell Sci.* **111,** 2867–2875.

Mah, S. P., Saueressig, H., Goulding, M., Kintner, C., and Dressler, G. R. (2000). Kidney development in cadherin-6 mutants: Delayed mesenchyme-to-epithelial conversion and loss of nephrons. *Dev. Biol.* **223,** 38–53.

Majumdar, A., Lun, K., Brand, M., and Drummond, I. A. (2000). Zebrafish no isthmus reveals a role for pax2.1 in tubule differen-

© 2002 Elsevier Science (USA). All rights reserved.

tiation and patterning events in the pronephric primordia. *Development* **127,** 2089–2098.
Marcussen, N. (1995). Atubular glomeruli in chronic renal disease. *Curr. Top. Pathol.* **88,** 145–171.
Marquardt, T., Ashery-Padan, R., Andrejewski, N., Scardigli, R., Guillemot, F., and Gruss, P. (2001). Pax6 is required for the multipotent state of retinal progenitor cells. *Cell* **105,** 43–55.
Mendelsohn, C., Batourina, E., Fung, S., Gilbert, T., and Dodd, J. (1999). Stromal cells mediate retinoid-dependent functions essential for renal development. *Development* **126,** 1139–1148.
Miller, C., and Sassoon, D. A. (1998). Wnt-7a maintains appropriate uterine patterning during the development of the mouse female reproductive tract. *Development* **125,** 3201–3211.
Miner, J. H., Patton, B. L., Lentz, S. I., Gilbert, D. J., Snider, W. D., Jenkins, N. A., Copeland, N. G., and Sanes, J. R. (1997). The laminin alpha chains: Expression, developmental transitions, and chromosomal locations of alpha1–5, identification of heterotrimeric laminins 8–11, and cloning of a novel alpha3 isoform. *J. Cell Biol.* **137,** 685–701.
Miner, J. H., and Li, C. (2000). Defective glomerulogenesis in the absence of laminin alpha5 demonstrates a developmental role for the kidney glomerular basement membrane. *Dev. Biol.* **217,** 278–289.
Naka, T., Fujimoto, M., and Kishimoto, T. (1999). Negative regulation of cytokine signaling: STAT-induced STAT inhibitor *Trends Biochem. Sci.* **24,** 394–398.
Neaves, W. B. (1973). Permeability of Sertoli cell tight junctions to lanthanum after ligation of ductus deferens and ductuli efferentes. *J. Cell Biol.* **59,** 559–572.
Neubauer, B. L., Chung, L. W., McCormick, K. A., Taguchi, O., Thompson, T. C., and Cunha, G. R. (1983). Epithelial–mesenchymal interactions in prostatic development. II. Biochemical observations of prostatic induction by urogenital sinus mesenchyme in epithelium of the adult rodent urinary bladder. *J. Cell Biol.* **96,** 1671–1676.
Nordling, S., Miettinen, H., Wartiovaara, J., and Saxen, L. (1971). Transmission and spread of embryonic induction. I. Temporal relationships in transfilter induction of kidney tubules in vitro. *J. Embryol. Exp. Morphol.* **26,** 231–252.
Obara-Ishihara, T., Kuhlman, J., Niswander, L., and Herzlinger, D. (1999). The surface ectoderm is essential for nephric duct formation in intermediate mesoderm. *Development* **126,** 1103–1108.
Okada, H., Inoue, T., Suzuki, H., Strutz, F., and Neilson, E. G. (2000). Epithelial-mesenchymal transformation of renal tubular epithelial cells in vitro and in vivo. *Nephrol. Dial. Transplant.* **15**(Suppl. 6), 44–46.
Perantoni, A. O., Dove, L. F., and Karavanova, I. (1995). Basic fibroblast growth factor can mediate the early inductive events in renal development. *Proc. Natl. Acad. Sci. USA* **92,** 4696–4700.
Plisov, S. Y., Yoshino, K., Dove, L. F., Higinbotham, K. G., Rubin, J. S., and Perantoni, A. O. (2001). TGFβ2, LIF and FGF2 cooperate to induce nephrogenesis. *Development* **128,** 1045–1057.
Potten, C. S., and Morris, R. J. (1988). Epithelial stem cells in vivo. *J. Cell Sci. Suppl.* **10,** 45–62.
Quaggin, S. E., Schwartz, L., Cui, S., Igarashi, P., Deimling, J., Post, M., and Rossant, J. (1999). The basic-helix–loop–helix protein pod1 is critically important for kidney and lung organogenesis. *Development* **126,** 5771–5783.
Quaggin, S. E., Van den Heuvel, G. B., and Igarashi, P. (1998). Pod-1, a mesoderm-specific basic-helix–loop–helix protein expressed in mesenchymal and glomerular epithelial cells in the developing kidney. *Mech. Dev.* **71,** 37–48.
Rothenpieler, U. W., and Dressler, G.(1993). Pax-2 is required for mesenchyme-to-epithelium conversion during kidney development. *Development* **119,** 711–720.
Saxen, L. (1987). "Organogenesis of the Kidney." Cambridge Univ. Press, Cambridge.
Schedl, A., and Hastie, N. D. (2000). Cross-talk in kidney development. *Curr. Opin. Genet. Dev.* **10,** 543–549.
Schuchardt, A., D'Agati, V., Larsson-Blomberg, L., Costantini, F., and Pachnis, V. (1994). Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret. *Nature* **367,** 380–383.
Sheng, Z., Pennica, D., Wood, W. I., and Chien, K. R. (1996). Cardiotrophin-1 displays early expression in the murine heart tube and promotes cardiac myocyte survival. *Development* **122,** 419–428.
Socolovsky, M., Lodish, H. F., and Daley, G. Q. (1998). Control of hematopoietic differentiation: Lack of specificity in signaling by cytokine receptors. *Proc. Natl. Acad. Sci. USA* **95,** 6573–6575.
Spooner, B. S., and Wessells, N. K. (1970). Mammalian lung development: Interactions in primordium formation and bronchial morphogenesis. *J. Exp. Zool.* **175,** 445–454.
Stark, M. R., Sechrist, J., Bronner-Fraser, M., and Marcelle, C. (1997). Neural tube-ectoderm interactions are required for trigeminal placode formation. *Development* **124,** 4287–4295.
Stark, K., Vainio, 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.
Vainio, S., and Muller, U. (1997). Inductive tissue interactions, cell signaling, and the control of kidney organogenesis. *Cell* **90,** 975–978.
Vainio, S., Jalkanen, M., Bernfield, M., and Saxen, L. (1992). Transient expression of syndecan in mesenchymal cell aggregates of the embryonic kidney. *Dev. Biol.* **152,** 221–231.
Vestweber, D., Kemler, R., and Ekblom, P. (1985). Cell-adhesion molecule uvomorulin during kidney development. *Dev. Biol.* **112,** 213–221.
Walther, C., and Gruss, P. (1991). Pax-6, a murine paired box gene, is expressed in the developing CNS. *Development.* **113,** 1435–1449.
Wu, H., Liu, X., Jaenisch, R., and Lodish, H. F. (1995). Generation of committed erythroid BFU-E and CFU-E progenitors does not require erythropoietin or the erythropoietin receptor. *Cell* **83,** 59–67.

Received for publication September 5, 2001
Revised March 1, 2002
Accepted March 4, 2002
Published online May 7, 2002

© 2002 Elsevier Science (USA). All rights reserved.