Roles of Hepatocyte Growth Factor/Scatter Factor and the Met Receptor in the Early Development of the Metanephros

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Abstract. Several lines of evidence suggest that hepatocyte growth factor/scatter factor (HGF/SF), a soluble protein secreted by embryo fibroblasts and several fibroblast lines, may elicit morphogenesis in adjacent epithelial cells. We investigated the role of HGF/SF and its membrane receptor, the product of the c-*met* protooncogene, in the early development of the metanephric kidney. At the inception of the mouse metanephros at embryonic day 11, HGF/SF was expressed in the mesenchyme, while met was expressed in both the ureteric bud and the mesenchyme, as assessed by reverse transcription PCR, in situ hybridization, and immnuohistochemistry. To further investigate the expression of met in renal mesenchyme, we isolated 13 conditionally immortal clonal cell lines from transgenic mice expressing a temperature-sensitive mutant of the SV-40 large T antigen. Five had the HGF/SF$^+$/met$^+$ phenotype and eight had the HGF/SF$^-$/met$^+$ phenotype. None had the HGF/SF$^+$/met$^-$ nor the HGF/SF$^-$/met$^-$ phenotypes. Thus the renal mesenchyme contains cells that express HGF/SF and met or met alone. When metanephric rudiments were grown in serum-free organ culture, anti-HGF/SF antibodies (*a*) inhibited the differentiation of metanephric mesenchymal cells into the epithelial precursors of the nephron; (*b*) increased cell death within the renal mesenchyme; and (*c*) perturbed branching morphogenesis of the ureteric bud. These data provide the first demonstration for coexpression of the HGF/SF and met genes in mesenchymal cells during embryonic development and also imply an autocrine and/or paracrine role for HGF/SF and met in the survival of the renal mesenchyme and in the mesenchymal–epithelial transition that occurs during nephrogenesis. They also confirm the postulated paracrine role of HGF/SF in the branching of the ureteric bud.

Two types of interaction between epithelial and mesenchymal cells are widespread during mammalian development (Bard, 1990). First, the growth of embryonic epithelia is often dependent on adjacent mesenchymal cells. Using tissue dissociation techniques, Grobstein (1967) demonstrated that the embryonic epithelia of the kidney, lung, salivary gland, and pancreas failed to undergo branching morphogenesis if separated from their native mesenchymes, whereas epithelial growth and differentiation was normal when the components were recombined in vitro. Secondly, conversions between the two types of tissue can occur and a transition from a mesenchymal to an epithelial phenotype occurs during somite formation, vasculogenesis, and nephrogenesis (Bard and Ross, 1991). It is a major goal of developmental biology to identify the molecular mechanisms that mediate these tissue interactions (Birchmeier and Birchmeier, 1993).

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The mammalian metanephros develops into the adult kidney and provides an excellent model for the study of mesenchymal/epithelial interactions (Saxen, 1987; Bard, 1991; Hardman et al., 1994*a*; Fig. 1). At the inception of the mouse metanephros on embryonic day 11 (E11),[1] the ureteric bud, which is comprised of polarized cytokeratin-expressing epithelial cells, contacts the nephrogenic mesenchyme and thereafter branches to form the arborial collecting duct system that drains urine from the nephron tubules (Fig. 1, *A* and *E*). Simultaneously, vimentin-expressing mesenchymal cells that lie adjacent to the branching tips of the bud differentiate into the cytokeratin-positive epithelia of the nephrons (Lehtonen et al., 1985) or into interstitial fibroblasts (Weller et al., 1991; Herzlinger et al., 1992). Other mesenchymal cells die by apoptosis, or programmed cell death (Koseki et al., 1992; Coles et al., 1993). On the first day after contact by the ureteric bud (E11–E12), renal mesen-

1. *Abbreviations used in this paper*: E11, embryonic day 11; HGF/SF, hepatocyte growth factor/scatter factor; RT-PCR, reverse transcription PCR; SV-40 T Ag, SV-40 T antigen.

chymal cells become induced to differentiate (Saxen, 1987), and they then express syndecan, a cell surface proteoglycan (Vainio et al., 1989). The cells that are destined to convert into nephrons aggregate around the branching tips of the ureteric bud to form condensations that express uvomorulin (E-cadherin) (Vestweber and Kemler, 1985; Klein et al., 1988; Fig. 1 *F*). From E13 onwards, the cells in the condensates develop an apical and basolateral polarity to form immature epithelia (Fig. 1, *C* and *G*, *comma- and S-shaped bodies*) that subsequently differentiate into nephrons. The proximal ends of the nephrons later differentiate into the epithelial podocyte layer that filters the plasma (Figs. 1, *D* and *H*). Throughout nephrogenesis, sequential layers of nephrons are born with the most immature elements located at the periphery of the organ.

Recent descriptive and in vitro studies of kidney organogenesis have suggested that a variety of molecules play a role in mesenchymal-driven epithelial growth, as well as in the differentiation of mesenchymal into epithelial cells. These molecules include transcription factors (Rothenpieler and Dressler, 1993), cell surface and extracellular matrix components (Platt et al., 1987; Sariola et al., 1988; Klein et al., 1988), and cytokines and their receptors (Sariola et al., 1991; Rogers et al., 1991, 1992). Cytokines, including EGF, have also been implicated in enhancing survival of renal mesenchyme (Koseki et al., 1992) and the growth of interstitial stromal cells (Weller et al., 1991) which, with nephron epithelia, have a common cellular origin in the renal mesenchyme (Herzlinger et al., 1992).

Early experiments with cells in culture (Stoker et al., 1987) and recent studies of expression patterns in mouse embryos (Sonnenberg et al., 1993) have suggested that the fibroblast-derived protein known as hepatocyte growth factor/scatter factor (HGF/SF) may act as an effector of mesenchymal/epithelial interactions. HGF/SF is secreted by mesenchymal cells and, in vitro, it induces epithelial cell movement (Stoker et al., 1987), proliferation (Nakamura et al., 1989), and branching morphogenesis (Montesano et al., 1991). All these effects are mediated by a specific membrane receptor, the tyrosine kinase encoded by the c-*met* protooncogene (Bottaro et al., 1991; Naldini et al., 1991; Weidner et al., 1993). After binding of HGF/SF with met, receptor phosphorylation is accompanied by an interaction with SH2- (Src homology 2 domains) containing intracellular signal transducers (Ponzetto et al., 1994). It was recently reported that HGF/SF transcripts are located in the nephrogenic mesenchyme, while met is expressed in the ureteric bud and its branches, as well as in epithelial cells of the nephron (Sonnenberg et al., 1993). The data strongly suggested that HGF/SF acts as a cytokine produced by mesenchymal cells that elicits the growth and differentiation of the adjacent epithelia in the ureteric bud and primitive nephrons (Sonnenberg et al., 1993).

When the early mouse metanephros is grown in organ culture, tissue differentiation proceeds during 2–4 d (Saxen, 1987). Moreover, the ability of the metanephros to grow in defined, serum-free media has allowed the roles of endogenously produced growth factors to be investigated. In the current study, we have investigated the role of HGF/SF in the interactions of mesenchymal and epithelial cells in early nephrogenesis by blocking HGF/SF in serum-free E11 and E12 metanephric organ culture with anti-HGF/SF antibodies. Additionally, to characterize the specific cell types that may express HGF/SF and met, we derived conditionally immortal clonal lines from E11 metanephric mesenchyme of the H-2K^b-tsA58 transgenic mouse, which harbors a γ-interferon–inducible temperature-sensitive mutant of the SV-40 T antigen (SV-40 T Ag) (Jat et al., 1991; Whitehead et al., 1993). These data provide the first demonstration for coexpression of the HGF/SF and met genes in mesenchymal cells during embryonic development and also imply a paracrine or autocrine role for HGF/SF and met in the proliferation and survival of the renal mesenchyme and in the mesenchymal-epithelial transition that occurs during nephrogenesis. They also confirm the postulated paracrine role of HGF/SF in the branching of the ureteric bud.

Materials and Methods

Reverse Transcription PCR for HGF/SF and Met

RNA was extracted from embryonic kidneys and transgenic cell clones by the acid phenol-chloroform method (Chomczynski and Sacchi, 1987), and 300 ng of RNA was subjected to reverse transcription and PCR amplification. The primers used were as follows: for met, 5′-GAA TGT CGT CCT ACA CGG CC-3′ (sense primer corresponding to nt 159-179) and 5′-CAG GGG CAT TTC CAT GTA GG-3′ (antisense primer corresponding to nt 866-886 of mouse c-*met* cDNA, Chan et al., 1987); for HGF/SF, 5′-TT GGC CAT GAA TTT GAC CTC-3′ (sense primer corresponding to nt 338-358) and 5′-AC ATC AGT CTC ATT CAC AGC-3′ (antisense primer corresponding to nucleotides 876-896 of rat HGF/SF cDNA; Tashiro et al., 1990). Primers for β-actin were obtained from Clontech (Palo Alto, CA). RNA was incubated with 10 μM 3′ primer for 10 min at 65°C and then reverse transcribed for 1 h at 42°C with 160 U Moloney murine leukemia virus reverse transcriptase in first-strand cDNA synthesis buffer with 1.25 mM each of dATP, dCTP, dGTP, and dTTP, 20 U RNase inhibitor, and 10 mM dithiothreitol. Negative controls contained no RNA or no reverse transcriptase. After reverse transcription, 15 μM 5′ primer, 5 μM 3′ primer, 2.5 U Taq DNA polymerase, and polymerase buffer (Promega Corp., Madison, WI) were added and the reaction volume made up to 50 μl with Trypsin EDTA. The PCR machine (Quatro TC-40) was programmed for 30 cycles as follows: 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, and finally 72°C for 7 min. 7 μl of the reaction product was electrophoresed through a 1% agarose gel with ethidium bromide. These protocols resulted in the following products: 732 bp for met, 559 bp for HGF/SF, and 540 bp for β-actin.

In Situ Hybridization for HGF/SF and Met

Metanephric rudiments were fixed in ice-cold 4% paraformaldehyde overnight, washed in saline, dehydrated in ethanol, and cleared in xylene. They were then embedded in Fibrowax (BDH, Leicestershire, U.K.) and 6–8-μm sections were cut and placed on slides coated with aminopropylthiethoxysilane (Sigma Immunochemicals, St. Louis, MO). These were kept at 4°C until processing for in situ hybridization. RNA probes were prepared with ^{35}S-UTP (>1,000 Ci/mmol; Amersham International, Amersham, UK) as run-off transcripts of a full-length HGF/SF template and a 2.1-kb met template (Chan et al., 1988). In both cases, templates were subcloned in the vector pBluescript KS−, antisense probes were produced using T3 RNA polymerase after linearization with SpeI, and sense probes were generated using T7 RNA polymerase after linearization with ApaI (HGF/SF) and KpnI (met). Probes were purified through Sephadex G50 columns, hydrolyzed at 60°C for 90 min, and used at 100,000 cpm/slide. Hybridizations, washings, and autoradiography were carried out as described in Wilkinson et al. (1987).

Metanephric Organ Culture

The morning of the vaginal plug was defined as embryonic day 0 (E0). Nontransgenic mouse embryos (CBA/Ca × C57Bl/10) were harvested at E11 and E12 when the ureteric bud had respectively penetrated (Fig. 1, *A* and *E*) or branched once (Fig. 1, *B* and *F*) within the metanephric mesenchyme. Organs were explanted onto transparent, permeable supports (Millicell™ CM; Millipore Corp., Bedford, MA) and were cultured essentially as de-

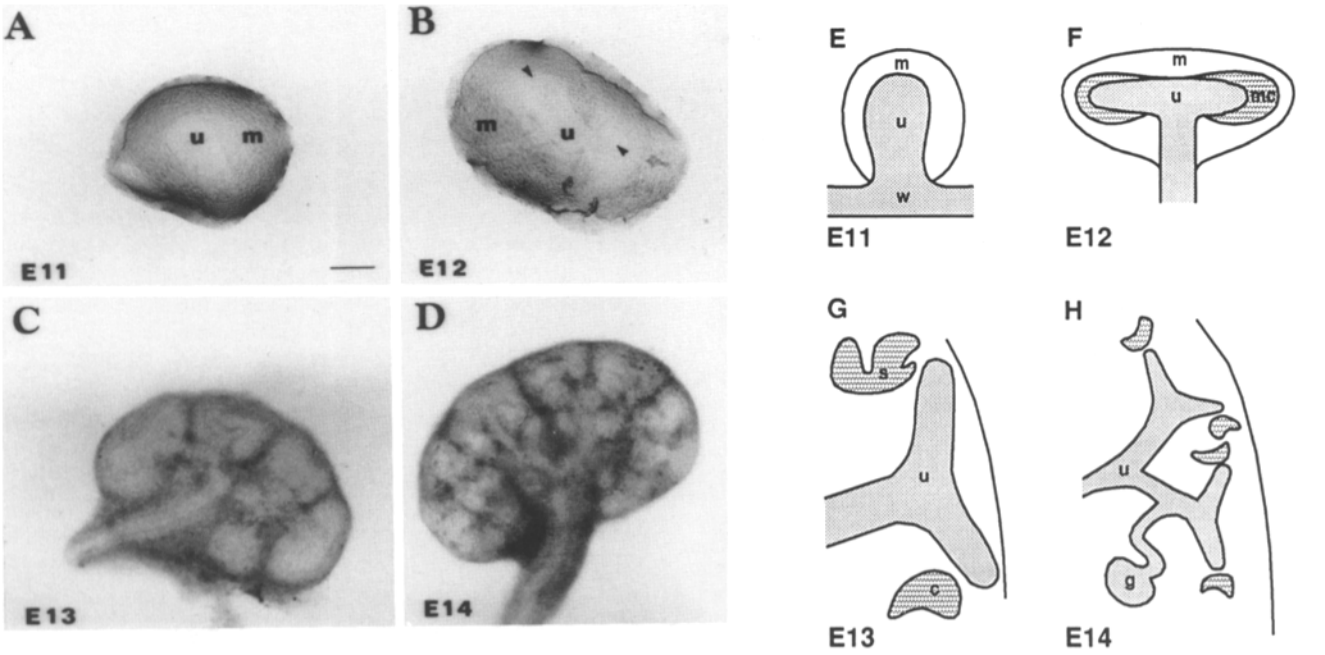


Figure 1. Early development of the metanephros. Stereomicroscopic images of the mouse metanephros (*A–D*) and cross-sectional diagrams of the metanephros (*E–H*) on E11 (*A* and *E*), E12 (*B* and *F*), E13 (*C* and *G*), and E14 (*D* and *H*). Note that the most primitive structures are located in the periphery of the E14 organ. Arrowheads in *B* indicate the first branch tips of the ureteric bud. *u*, ureteric bud; *m*, mesenchyme; *mc*, mesenchymal condensate; *c* and *s*, comma and S-shaped bodies, respectively; *g*, glomerulus; and *w*, Wolffian duct. Bar, 50 μm in *A–F*. *E–H* are not drawn to scale.

scribed in Hardman et al. (1993) in serum-free basal media at 37°C in a 5% CO_2/air humidified atmosphere. The basal medium consisted of Dulbecco's modified Eagle's medium/Ham's F-12 (GIBCO BRL, Gaithersburg, MD) supplemented with glutamine (1 mM) progesterone (1.5 μg/liter), insulin (1 mg/liter), putrescine (0.4 mg/liter), thyroxine (10 μg/liter), triiodothyronine (7 μg/liter), selenium (1 μg/liter), transferrin (100 μg/liter), penicillin G (1,000 U/liter), and streptomycin (1 mg/liter). 15 E11 and 30 E12 organs were grown for 72 h in each of three conditions: (*a*) basal media alone; (*b*) basal media and 10 mg/l IgG Fab_2 fragments of rabbit anti-mouse HGF/SF antibody to block the bioactivity of HGF/SF (FIB3D see "Bioassay for HGF/SF" below; Furlong et al., 1991); (*c*) basal media and 10 mg/l Fab_2 fragments prepared from nonimmune rabbit IgG (DAKO Ltd., Bucks, UK). The same concentration of anti-HGF/SF antibodiy was used in preliminary titration experiments to establish the ability of the anti-HGF/SF IgG to neutralize the activity of mouse recombinant HGF/SF on MDCK cells. At 500 and 250 pM HGF/SF, neither preimmune IgG nor anti-HGF/SF IgG had any effect. Anti-HGF/SF IgG, however, substantially inhibited the factor at the concentration between 7.8 and 125 pM, which is within the range of fibroblasts in culture (Stoker et al., 1987). In a limited set of experiments ($n = 5$ for each condition), the media were supplemented with 5% vol/vol heat-inactivated FBS. Media were changed daily and morphology was documented with photomicrographs using an inverted microscope. Ureteric bud branch tips were counted on day 3, expressed as median (range), and groups were compared using unpaired Wilcoxon Rank tests. On day 3, the organs were fixed for 2 h in 4% paraformaldehyde in PBS (pH 7.0) before being processed for 10-μm thick paraffin-embedded sections or for endogenous galactosidase activity by whole-mount histochemistry as described by Bard and Ross (1991). The latter technique uses the X-gal reagent to stain and identify mature renal epithelia.

Confocal Microscopy of Organ Culture Rudiments

Whole E11 metanephric rudiments were cultured as described above in the presence of (*a*) basal media alone; (*b*) basal media with 10 mg/l rabbit anti-mouse HGF/SF; (*c*) 10 mg/l control IgG; or (*d*) basal media with 100 pM recombinant HGF/SF. Antibodies were prepared as described below. Recombinant HGF/SF was purified by heparin-Sepharose CL 6B chromatography from the supernatant cultures of the mouse myeloma line NSO transfected with a full-length cDNA clone of mouse HGF/SF. After 48 h, the inserts were washed with PBS (pH 7.0), and 1.5 ml of ice-cold 4% paraformaldehyde in PBS was added underneath the insert. After overnight fixation at 4°C, the inserts were washed 3× in PBS, and the rudiments were stained with propidium iodide by a modification of the technique of Coles et al. (1993). Rudiments were exposed to 4 mg/liter propidium iodide (Sigma) and 100 mg/liter RNase (DNase free; Sigma) in PBS. After incubation in the dark for 2 h at 37°C, the inserts were washed with PBS to remove excess stain. The membrane with the rudiments was cut from the inserts and mounted on microscope slides with Citifluor (City University, London). Coverslips were sealed with nail varnish and slides were examined using a confocal microscope (CSLM; Leica Lasertechnik GmbH, Heidelberg, Germany). Unless otherwise stated, the ×25 objective was used with a zoom factor of 1. Within each rudiment, four areas were examined: two peripheral areas containing mesenchyme and nephron progenitors, as well as two central areas containing branches of the ureteric bud, mesenchyme, and nephron precursors. Before scanning, the depth of the rudiment was estimated and the confocal microscope was programmed to scan each area at three optical planes equivalent to the center of the organ and 10 μm on either side of the center. An image of each optical section was created by averaging eight frames. Using this technique, mitotic figures are easily visualized, as are apoptotic nuclei that appear small, irregular, and bright (Coles et al., 1993). Nuclei were counted and the results were expressed as mean ± SEM nuclei per area. The effects of different treatments were compared using Student's *t* test.

Metanephric Cell Lines

Homozygous male H-2K^b-tsA58 transgenic mice (Jat et al., 1991) were mated with nontransgenic females of the same strain (CBA/Ca × C57Bl/10). These along with nontransgenic embryos were harvested at E11 (Fig. 1, *A* and *E*). After careful microdissection of the renal mesenchyme away from the ureteric bud, fragments of mesenchyme, comprising 100–1,000 cells, from transgenic and nontransgenic embryos were placed into plastic wells (Nunc Inc., Roskilde, Denmark) coated with fibronectin (20 mg/liter in water; Sigma). Fibronectin was used as substrate because it is a major component of the E11–E12 metanephric mesenchyme matrix (Saxen, 1987). Explants were incubated in a humidified 5% CO_2/air atmosphere in the basal medium (see "Metanephric Organ Culture" above) with 1% vol/vol FBS. Nontransgenic cells were grown at 37°C without γ-interferon (nonpermissive conditions), while transgenic cells were initially expanded under permissive conditions; these were 33°C with recombinant murine γ-interferon

(40×10^3U/liter; Genentech, South San Francisco, CA). Transgenic cells were subsequently passaged after enzymatic dissociation (1,000 U/ml trypsin, 0.02% EDTA in Dulbecco's modified Eagle's medium for 5 min at room temperature) and then cloned by limiting dilution. Of 13 lines analyzed for HGF/SF and met expression by reverse transcription PCR (RT-PCR), two representative clones were selected for further study. The proliferation of these two clones was assessed by plating 10^3 cells into each well of a 24-well plate (Nunc) and counting viable cells that excluded trypan blue at 4, 7, and 11 d. Experiments were performed to determine cell proliferation under permissive and nonpermissive conditions in the basal media alone and when this was supplemented with either 1% FBS, 50 mg/liter insulin-like growth factor I (IGFI) (Genentech), or 1 nM recombinant mouse HGF/SF.

Bioassay for HGF/SF

Confluent monolayers of transgenic renal mesenchyme cells were washed twice in serum-free media and covered in basal medium with 1% vol/vol FBS. After 2 d, the conditioned medium was harvested, and HGF/SF scattering bioactivity was measured using monolayers of the MDCK epithelial cell line as a target (Stoker and Perryman, 1985). Briefly, serial doubling dilutions of the conditioned medium (0.15 ml) were added to a suspension of 3×10^3 MDCK cells in 0.15 ml DME with 5% vol/vol FBS in 96-well plates. After 18 h, the ability of the conditioned medium to prevent the formation of epithelial islands was recorded. The conditioned medium from a mouse cell line, D4-*ras* NIH3T3, which secretes high levels of HGF/SF, was used as a positive control (Gherardi et al., 1989). In addition, before the assay, duplicate samples were incubated for 4 h at 4°C with rabbit polyclonal anti-mouse HGF/SF antibody (FIB3D, IgG fraction, 10 mg/liter) (Furlong et al.,1991) to block bioactivity or with the same protein concentration of the IgG fraction of rabbit preimmune serum (DAKO). In some experiments, Fab_2 fragments were used. These were prepared by digestion of the IgG fractions with pepsin in 0.1 M sodium citrate for 1 h at 37°C at an antibody/enzyme ratio of 100:1. Digestion was terminated by addition of 3 M Tris-Cl, pH 8.5, and the Fab_2 fragments were purified by FPLC on a Superose 12 column equilibrated in PBS. Antibody concentration was determined by A_{280}.

Western Blotting for HGF/SF

Conditioned media from transgenic cell lines was concentrated 20 times with ammonium sulphate (80% saturation), resuspended in 10 mM Tris-Cl, pH 8.0, and electrophoresed in 8% polyacrylamide gels (Laemmli, 1970). Proteins were then transferred electrophoretically onto polyvinyldifluoride membranes in 10 mM CAPS, pH 12, essentially as described (Matsudaira, 1987). Membranes were blocked in 2% BSA for 2 h before overnight incubation with the anti-HGF/SF antibody (Furlong et al., 1991), which was detected with horseradish peroxidase-conjugated anti-rabbit antibody (DAKO).

Immunochemistry

10^3 cells were seeded into each fibronectin-coated well of eight-chamber slides (Nunc) and were grown for 1 wk under permissive conditions (33°C with IFN-γ). 10^4 cells were plated and cultured under nonpermissive conditions (39°C without IFN-γ) to achieve a similar degree of confluence after 1 wk. Cells were washed twice in PBS and fixed for 5 min with either 2% paraformaldehyde in PBS at room temperature or with ice-cold 100% ethanol before detection of surface or intracellular antigens, respectively. After a blocking step (incubation for 1 h at room temperature with 10% goat serum), cells were incubated at 4°C with the primary antibodies that were detected with appropriate FITC-conjugated second antibodies (DAKO). Primary antibodies were: mouse antipancytokeratin (Sigma), goat anti-human HGF/SF (R & D Systems, Inc., Minneapolis, MN), rabbit anti-mouse met (NBS Biologicals, Hatfield, Herts, U.K.), mouse anti-SV-40 T Ag (PAb 412 from Harlow et al., 1981), rat monoclonal anti-mouse syndecan (281-2 from Saunders et al., 1989), rat monoclonal antiuvomorulin (DECMA-1 from Vestweber and Kemler, 1985), and mouse monoclonal antivimentin (1112457; Boehringer Mannheim Biochemicals, Indianapolis, IN). The specificity of the anti-met antibody was confirmed by preincubation with a met control peptide (Santa Cruz Biotechnology). To localize met and HGF/SF protein within the metanephros, 10-μm cryostat sections of mouse kidneys were reacted with the above antibodies using the same protocols as for the cell lines, except that the sections were not fixed. Note that for HGF/SF immunohistochemistry, the anti-human antibody was used because it resulted in a stronger signal than the anti-mouse antibody that we used in other experiments.

Results

HGF/SF and met Expression in Early Nephrogenesis

As assessed by RT-PCR, HGF/SF and met transcripts were detected throughout early nephrogenesis (E11–E14), as well as in the kidneys of neonatal (postnatal day 1 [P1]) and adult mice (P90) (Fig. 2). In addition, using the same methodology, isolated E11 mesenchyme expressed both HGF/SF and met (Fig. 2). Using in situ hybridization, HGF/SF transcripts were detected in the metanephric mesenchyme on E11 (Fig. 3 *A*), and on E14 (Fig. 3 *D*), they appeared in a thin rim in the periphery of the metanephros where undifferentiated mesenchymal cells and renal capsular cells are located. HGF/SF mRNA was also detected on E14 in the interstitial tissues in the hilum of the kidney (not shown). On E11, HGF/SF protein was located between metanephric mesenchymal cells but not over the ureteric bud (Fig. 4 *C*), and on E14, both mesenchymal cells and primitive nephrons in the outer nephrogenic zone were surrounded by immunoreactive HGF/SF (Fig. 4 *D*). The more extensive distribution of the HGF/SF protein compared to mRNA is consistent with being a secreted protein that is sequestered in the extracellular matrix.

Using in situ hybridization, met transcripts were detected in the E11 metanephros both in the mesenchyme and the ureteric bud (Fig. 3 *B*). On E14, the highest expression of met was noted in the branching tips of the ureteric bud with lower activity in the renal mesenchyme and the primitive nephrons or S-shaped bodies (Fig. 3 *E*). Met mRNA was also detected on E14 in the epithelium of the ureter and its major branches (not shown). On E11, met protein was clearly identified in both the ureteric bud and also in the surrounding renal mesenchyme (Fig. 4 *A*), while on E14, it was detected in the

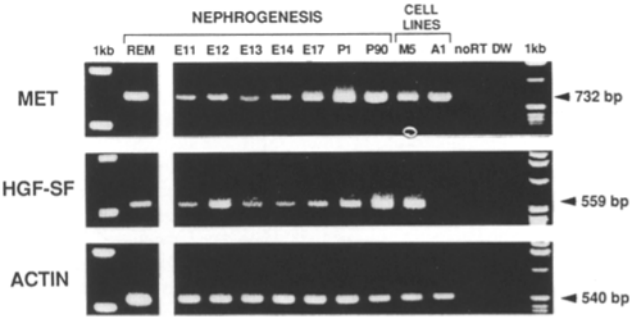


Figure 2. RT-PCR of HGF/SF and met during nephrogenesis and in mesenchymal cell lines. RT-PCR products for met, HGF/SF, and β-actin were located at 732, 559, and 540 bp, respectively. Met and HGF/SF transcripts were detected in isolated E11 renal mesenchyme (*REM*), in the whole metanephros during early (*E11–E14*) and late (*E17*) nephrogenesis, and in the whole kidney on postnatal days 1 and 90 (*P1* and *P90*). M5 and A1 are cell lines cloned from E11 metanephric mesenchyme. Note that met is expressed by both lines, but HGF/SF was only detected in the M5 clone. *1kb*, size markers; *no RT* and *DW*, negative controls (no reverse transcriptase and distilled water only, respectively).

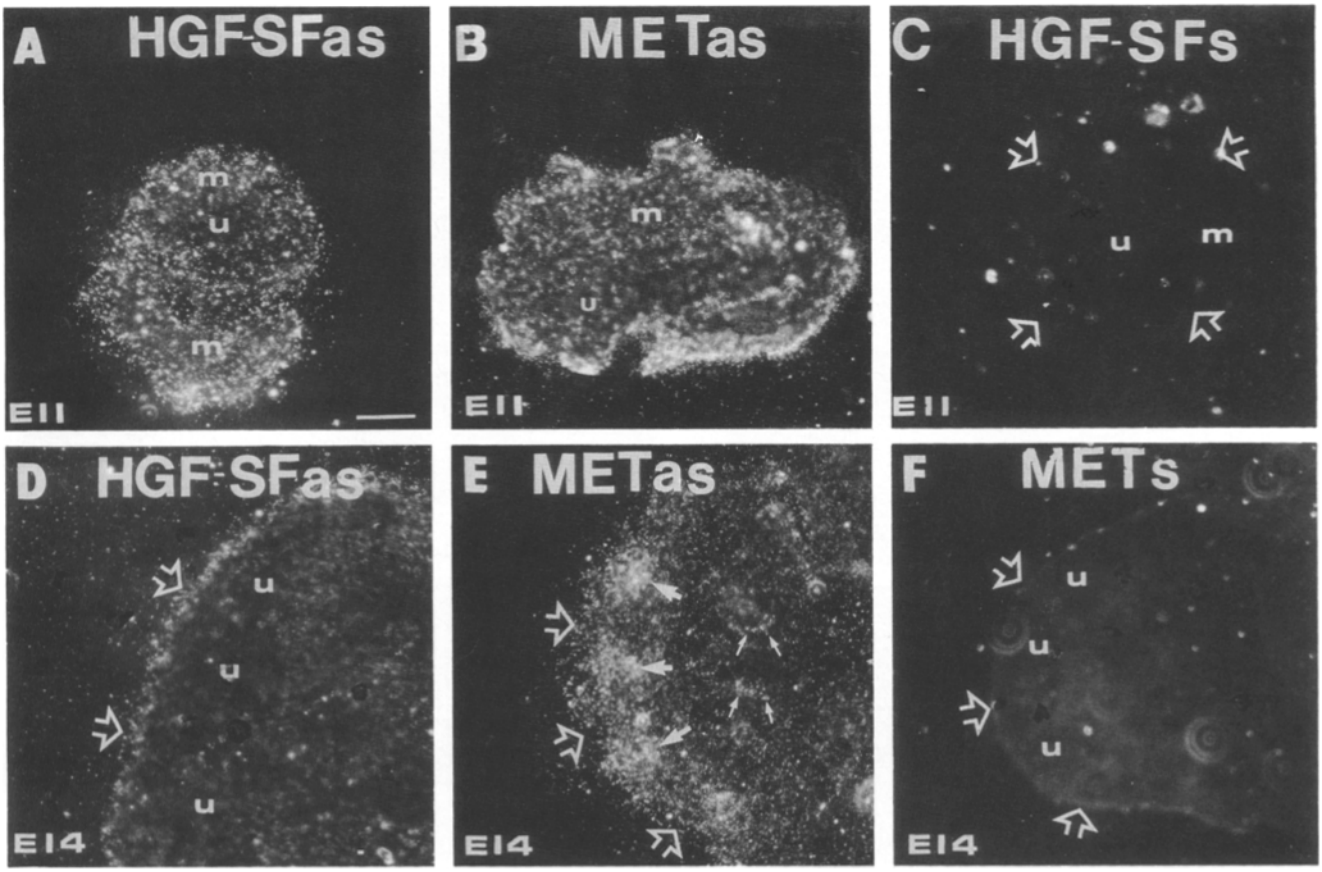


Figure 3. In situ hybridization for metanephric HGF/SF and met. (*A*) On E11, HGF/SF mRNA was detected in the nephrogenic mesenchyme. On E14 (*D*), HGF/SF transcripts were located in the periphery of the organ (*open arrows*), where stem cells and capsular cells are located, but not in the branch tips of the ureteric buds (*u*). Met transcripts were detected throughout the E11 organ (*B*). On E14 (*E*), met mRNA was highly expressed in the branching tips of the ureteric bud (*large closed arrows*). On E14, lower levels of met transcripts were also detected in the mesenchyme in the periphery of the organ (*open arrows*) and also in S-shaped bodies, which are primitive nephrons (*small closed arrows*). Sense riboprobes for HGF/SF (*C*) and met (*F*) reveal no significant hybridization; note lack of signal around the periphery of organs (*open arrows*). *m*, mesenchyme; *u*, the ureteric bud or its branches; *as*, antisense riboprobe and *s* indicates sense riboprobe. Bar, 50 μm.

branches of the ureteric bud, with lower levels in the nephrogenic zone (Fig. 4 *B*). On E14, the renal interstitium did not express met (Fig. 4 *B*).

Metanephric Mesenchymal Cell Lines

To investigate further the expression of met in the renal mesenchyme, we derived cell lines from the E11 metanephros. Nontransgenic E11 metanephric mesenchyme formed primary cultures but, in 10 separate experiments, it ceased to proliferate by the second passage. In contrast, transgenic cultures grown in the permissive conditions continued to proliferate, allowing clones to be isolated by limiting dilution. The clones maintained the same phenotypes for 25 passages and upon subcloning and thus far they been passaged more than 40 times, suggesting that they are immortal. When cultured in basal media with 1% vol/vol FBS, the continued proliferation of the clones was dependent on both γ-interferon and a temperature of 33°C. Removal of γ-interferon or shifting to a higher temperatures (37°C and 39.5°C) both resulted in a cessation of proliferation (Fig. 5). In addition, transgenic lines did not proliferate under nonpermissive conditions in the presence HGF/SF or IGFI. Immunostaining for SV-40 T Ag revealed that the protein was present only under the permissive condition (results not shown). Of 13 metanephric mesenchymal lines analyzed by RT-PCR and the MDCK bioassay, five were HGF/SF$^+$/met$^+$ and eight were HGF/SF$^-$/met$^+$. No clones were isolated with the HGF/SF$^-$/met$^-$ or HGF/SF$^+$/met$^-$ phenotypes. Two representative clones were selected for detailed investigation. These were the M5 (HGF/SF$^+$/met$^+$) and A1 (HGF/SF$^-$/met$^+$) lines (Figs. 2 and 6, *A* and *B*). Both expressed vimentin and syndecan (Fig. 6, *C* and *D*), but they did not stain with an anti-pancytokeratin antibody (not shown). From these results, we conclude that the M5 and A1 clones are induced renal mesenchymal cells, and not ureteric epithelial cells. The phenotypes of these lines were similar under permissive and nonpermissive conditions, except that the nonproliferating cells were larger.

The M5 cells had irregular outlines in monolayer culture (Fig. 6 *A*). They expressed HGF/SF as assessed by RT-PCR (Fig. 2). HGF/SF bioactivity was detected in M5-condi-

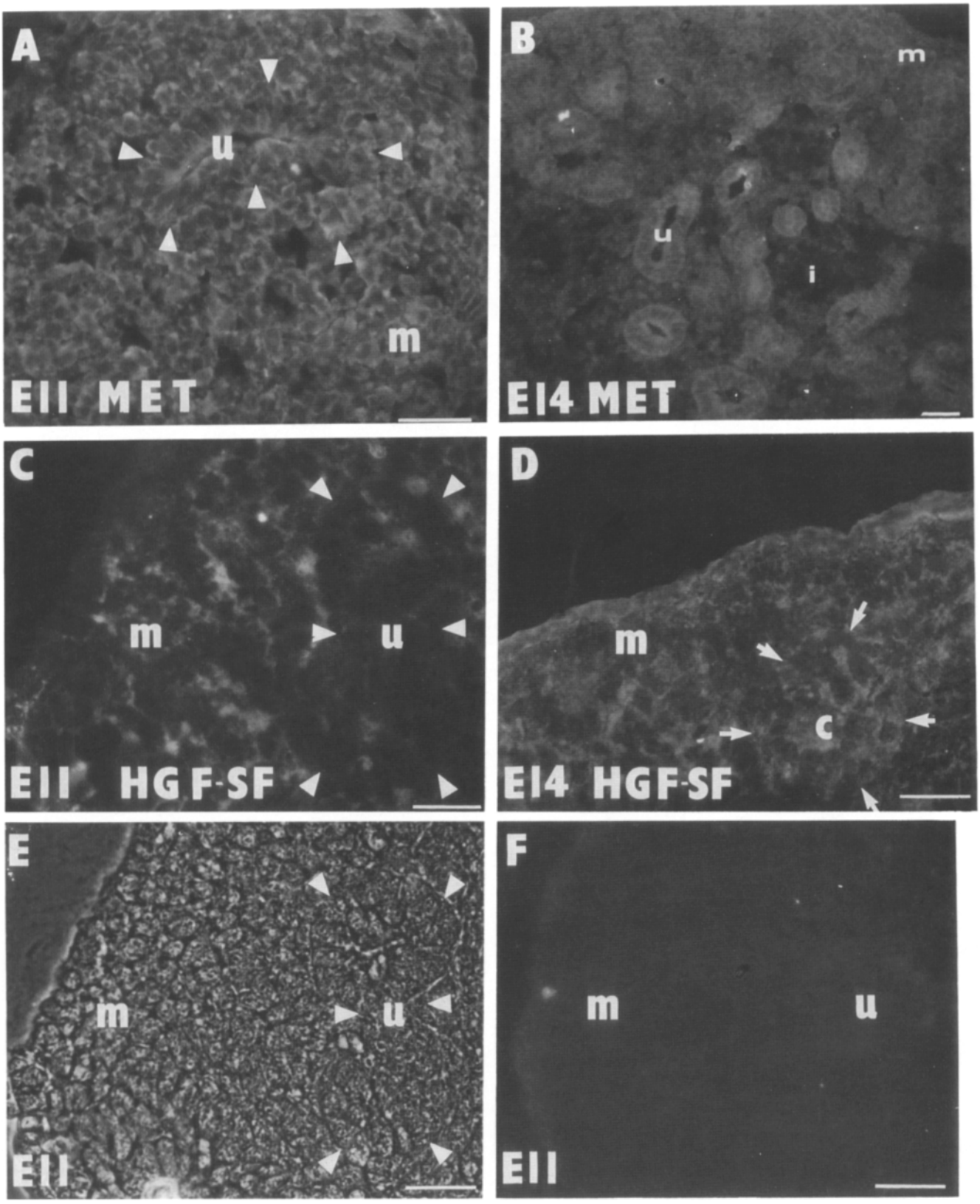


Figure 4. Immunohistochemistry for metanephric HGF/SF and met. (*A*) On E11, both the ureteric bud (*u*) and the mesenchyme (*m*) are positive for met. (*B*) On E14, strong met immunostaining is observed in the branches of the ureteric bud (*u*), and there is a weaker signal in the mesenchyme (*m*) in the periphery of the organ. At this stage, the renal interstitium (*i*) does not express met. (*C*) On E11, HGF/SF protein was detected in a patchy distribution in the nephrogenic mesenchyme, whereas the ureteric bud is negative. (*D*) On E14, HGF/SF immunoreactivity was located in the mesenchyme and around comma-shaped nephron precursors (*c*) in the periphery of the organ. Minimal background immunofluorescence was noted when the anti-met antibody was preabsorbed with met peptide (*F*). Bright fields of *C* and *F* is shown in *E*. The ureteric bud is indicated by arrowheads in *A*, *C*, and *E*. Bar, 25 μm.

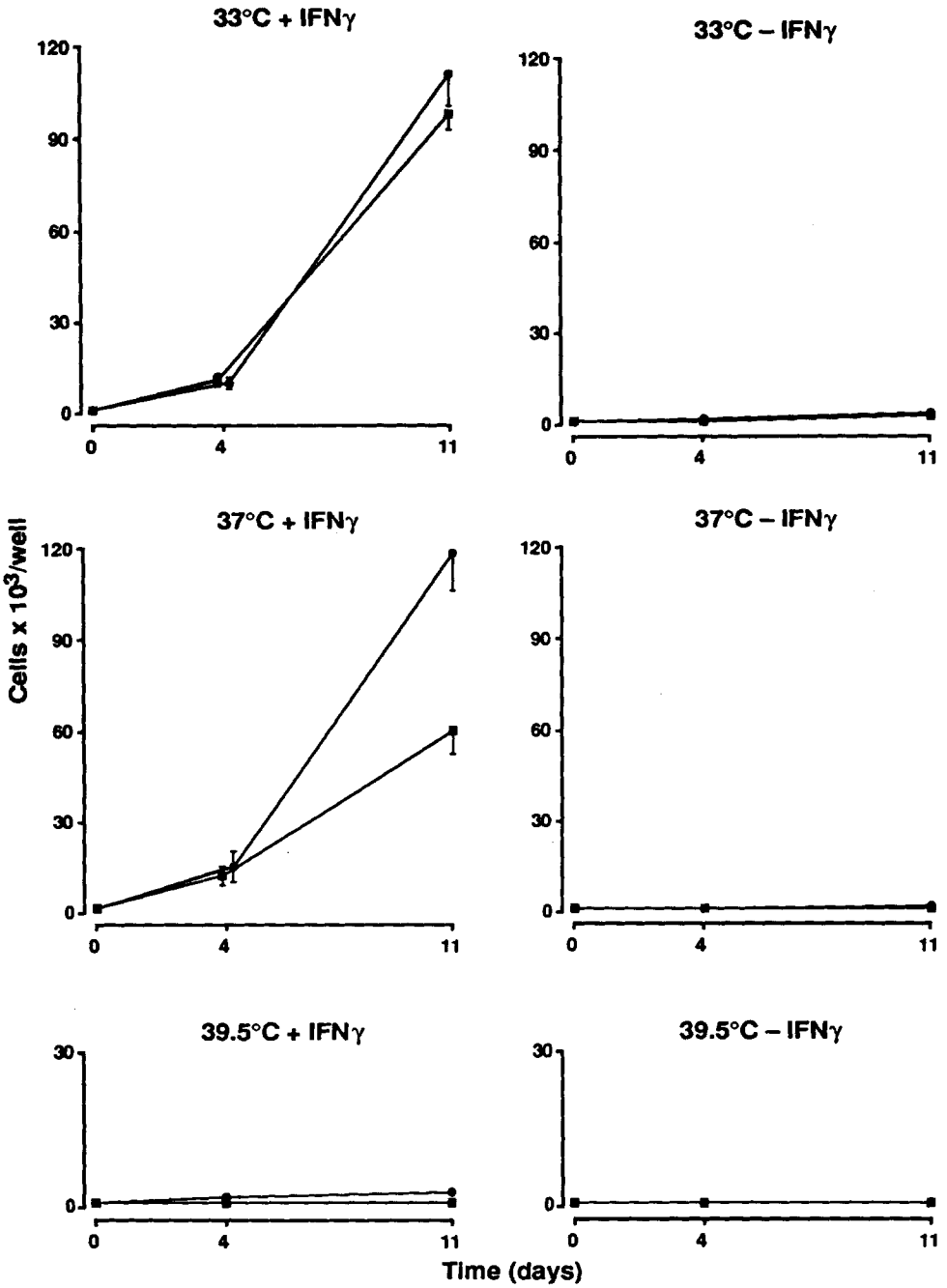


Figure 5. Proliferation of conditionally immortal renal mesenchyme lines. After seeding 10^3 of A1 or M5 cells onto fibronectin-coated dishes on day 0, they were grown at 33°C, 37°C, and 39°C with and without γ-interferon. Viable cells were counted at 4 and 11 d (n = 3, mean ± SD). ●, A1; ■, M5 lines.

tioned medium as assesed by the MDCK assay (one tenth the titer of a positive control *ras*-NIH 3T3 line), and HGF/SF immunoreactivity was detected by Western blotting (Fig. 7). The bioactivity of M5-condition medium could be abolished by preincubation with 10 mg/liter of the anti-mouse HGF/SF antibody (not shown). This cell line did not express uvomorulin (not shown), suggesting that it had not entered a mesenchymal to epithelial transition. In permissive conditions, both serum and IGFI significantly accelerated cell proliferation compared to the basal media, but HGF/SF did not enhance proliferation (Fig. 8), even though *met* transcripts were detected by RT-PCR (Fig. 2).

Individual A1 cells were cuboidal in shape and they had a cobblestone appearance at confluence (Fig. 6 *B*). They did not express HGF/SF as detected by RT-PCR (Fig. 2), and A1-conditioned media contained neither bioactive nor immunoreactive HGF/SF, as assessed by the MDCK scattering assay and Western blotting, respectively (Fig. 7). Met transcripts were detected by RT-PCR (Fig. 2), and the A1 cells stained positively with antibodies to met (not shown). In the permissive conditions, HGF/SF, IGFI, and FBS each significantly enhanced proliferation compared to the basal medium (Fig. 8). The A1 clonal line stained positively for uvomorulin (Fig. 6 *E*), suggesting that it had entered a mesenchymal to epithelial transition.

These results therefore establish that (*a*) conditionally immortal and phenotypically stable cell lines can be derived from the E11 mesenchyme; (*b*) these cell lines resemble cells

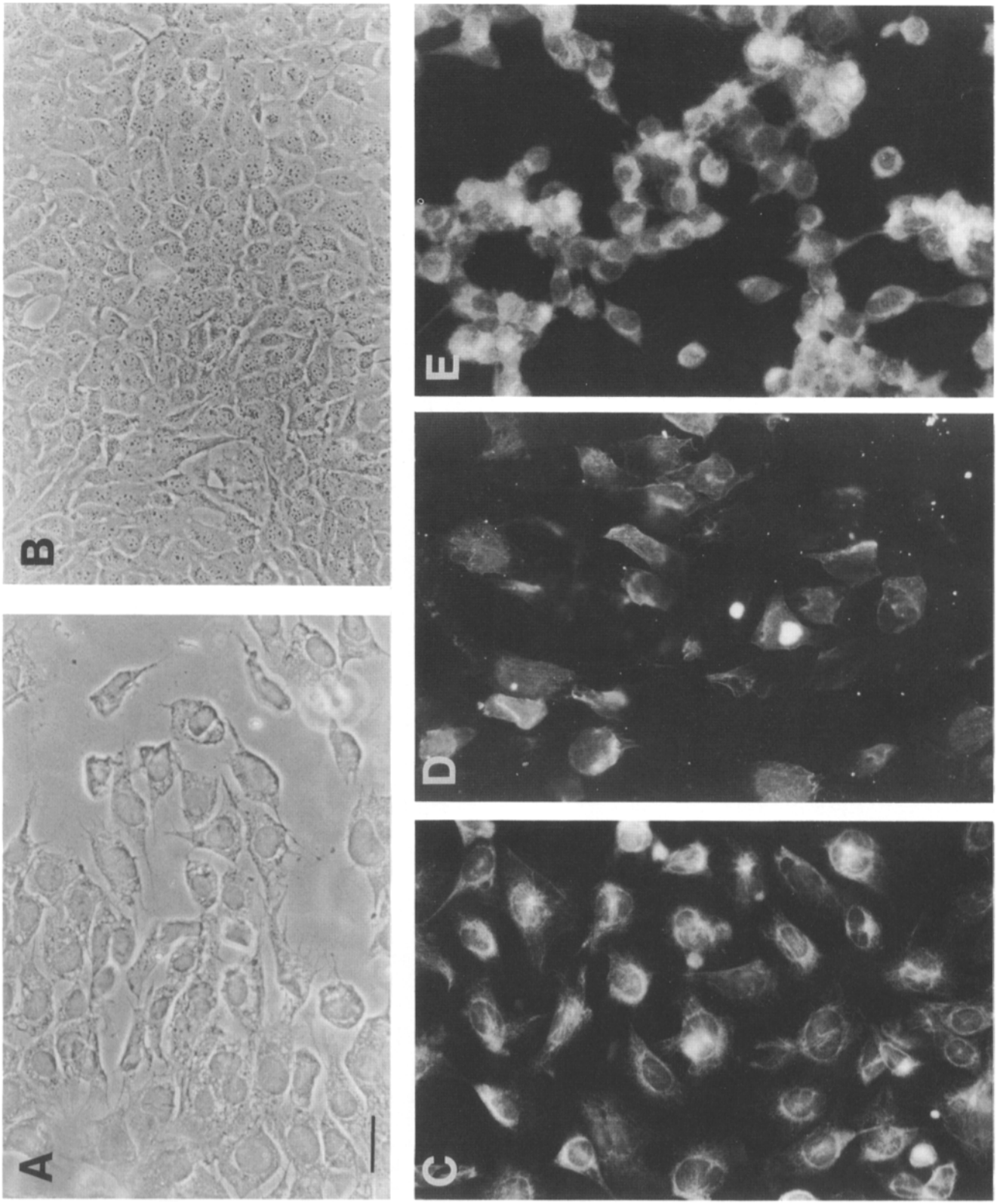


Figure 6. Phenotype of two metanephric mesenchymal cell lines. The M5 cells had an irregular "fibroblastic" outine in monolayer culture (*A*), while individual A1 cells were cuboidal and formed a compact cobblestone appearance at confluence (*B*). Positive immunostaining for vimentin (*C*) and syndecan (*D*) by the M5 clone; the A1 cells also stained with these antibodies (not shown). Only the A1 cells stained with antibodies to uvomorulin (*E*). Bar, 50 μm.

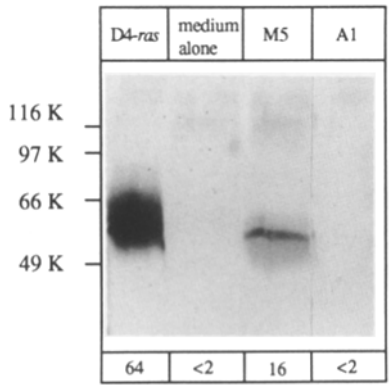


Figure 7. Western blotting for HGF/SF. Immunoreactivity for HGF/SF protein was detected at 62 kD in the conditioned media of D4 ras NIH 3T3 cells (positive control) and of the M5 clone, but it was not present in media from A1 or in unconditioned medium (medium alone). The titer of scattering activity from the MDCK bioassay is shown below each lane. Less than 2 indicates no detectable bioactivity.

at distinct stages of mesenchymal–epithelial transition; and (*c*) all cell lines express met and a subset coexpress HGF/SF and met.

Gross Effects of Anti-HGF/SF Antibody in Metanephric Organ Culture

When E11 organs were explanted, the ureteric bud had penetrated the mesenchyme but had not branched (Fig. 1, *A* and *E*). After 3 d of culture in basal media alone, (Fig. 9 *A*) or with nonimmune rabbit IgG Fab_2 (Fig. 9 *B*), the bud had divided to give a median of five branch tips (range = 2–10), and mesenchymal condensates were detected around the branches of the bud (Fig. 9 *D*). In contrast, in the presence of the anti-HGF/SF antibody (Fig. 9 *C*), branching was significantly limited compared to the controls, with only a median of 2 (range = 1–4) tips present after 3 d ($P < 0.05$). These branch tips were often dilated or cystic, and they were surrounded by mesenchyme that failed to form condensates (Fig. 9 *E*). With the anti-HGF/SF antibody, we also noted cell death in the mesenchyme, as assessed by the presence of darkly staining, pyknotic nuclei (Fig. 9 *E*). These appearances were suggestive of apoptosis and were investigated further as reported below (see "Confocal Microscopy of Metanephric Organ Culture" below). When E12 organs were explanted, the ureteric bud had branched once (Fig. 1, *B* and *F*), and after 3 d of organ culture a median of 10 (range = 6–17) branch tips had formed in basal media either alone (Fig. 9 *F*) or when nonimmune IgG was added (Fig. 9 *G*). Between the branches of the ureteric bud, X-gal-positive nephron precursors had developed (Fig. 9, *I* and *J*). Although branching of the ureteric bud did occur in E12 organs in the presence of the anti-HGF/SF antibody, the number of branch tips were reduced (median = 6, range = 3–12; $P < 0.05$ vs basal media alone; Fig. 9 *H*). Moreover, this treatment prevented the development of X-gal-positive nephron precursors (Fig. 9 *K*), suggesting a lack of mature nephron epithelia. Additional experiments demonstrated that concentrations of nonimmune Fab_2 IgG 10-fold greater that those used above did not inhibit nephrogenesis nor did they cause morphological evidence of cell death (data not shown).

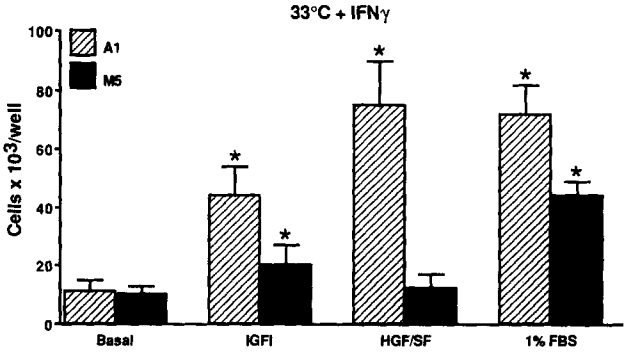


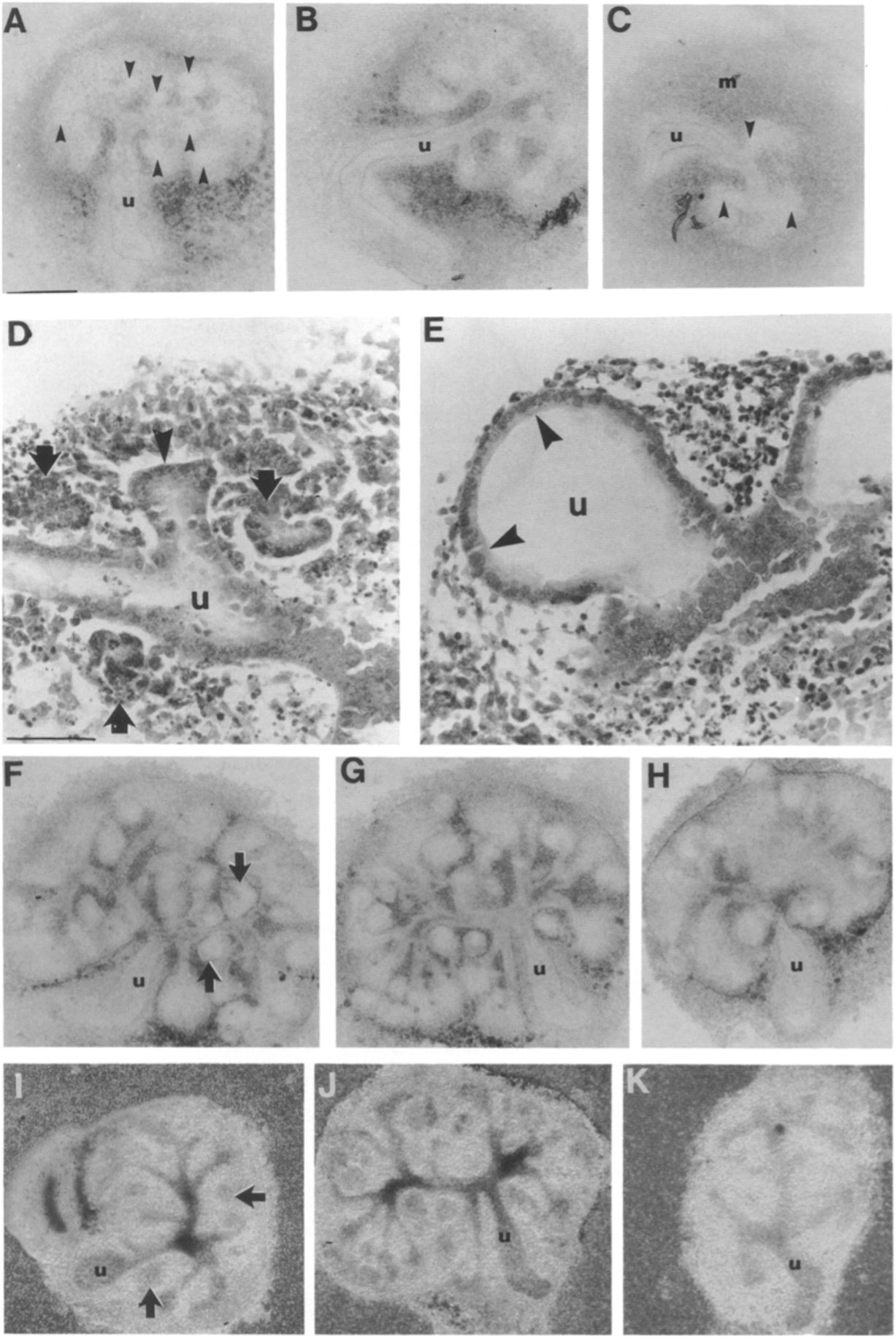
Figure 8. Effects of growth factors on proliferation of renal mesenchyme cell lines. 10^3 cells that were seeded on day 0 were grown for 11 d in permissive conditions (33°C with γ-interferon). After 11 d, viable cells were counted ($n = 4$, mean ± SD). *Significant difference from serum-free basal medium. HGF/SF and IGFI were added at 50 mg/liter.

Confocal Microscopy of Metanephric Organ Culture

We investigated the detailed tissue effects of manipulation of the HGF/SF/met axis using confocal microscopy of propidium iodide stained rudiments. At the time of explantation (E11), no primitive nephrons (comma or S-shaped bodies) were present. After 48 h in the basal media with the control antibody, comma-shaped nephron precursors had developed in the periphery of the organ, and more mature S-shaped bodies were evident in the presumptive renal medulla between the branches of the ureteric bud (Fig. 10, *A* and *C*). Organs cultured in basal medium alone showed a similar state of differentiation (not shown). In marked contrast, when organs were cultured with 10 mg/liter of the anti-HGF/SF antibody, there was little or no evidence of a mesenchymal to epithelial transition (Fig. 10, *B* and *D*). Compared to organs cultured in control antibody, the anti-HGF/SF antibody caused significant increase in the incidence of apoptotic nuclei after 48 h of culture in the center of the organ (Table I and Fig. 10 *D*): visual inspection revealed that apoptosis was prominent in the mesenchymal compartment rather than in the ureteric epithelium. The addition of recombinant HGF/SF (100 pM) did not produce significant morphological changes (not shown), nor did the factor significantly alter the frequency of apoptosis (Table I). Detailed analysis, however, revealed that HGF/SF caused a small increase in the incidence of mitotic figures in the peripheral mesenchyme (2.06 ± 0.22 vs 1.50 ± 0.18 mitoses per field, $P = 0.05$).

Discussion

In this study, we have investigated a putative role for HGF/SF and its receptor, met, in kidney development. Earlier experiments had suggested, based on the mRNA expression patterns of HGF/SF and met during kidney development, that HGF/SF derived from the renal mesenchyme could play a role in the development of the adjacent ureteric epithelia of the kidney (Sonnenberg et al., 1993). Consistent with this hypothesis, we report that antibodies to HGF/SF inhibit branching of the ureteric bud in organ cultures from E11 and E12 metanephros (Fig. 9). Our results also suggest that HGF/SF and met may be involved in the early development of the nephron because the formation of nephron precursors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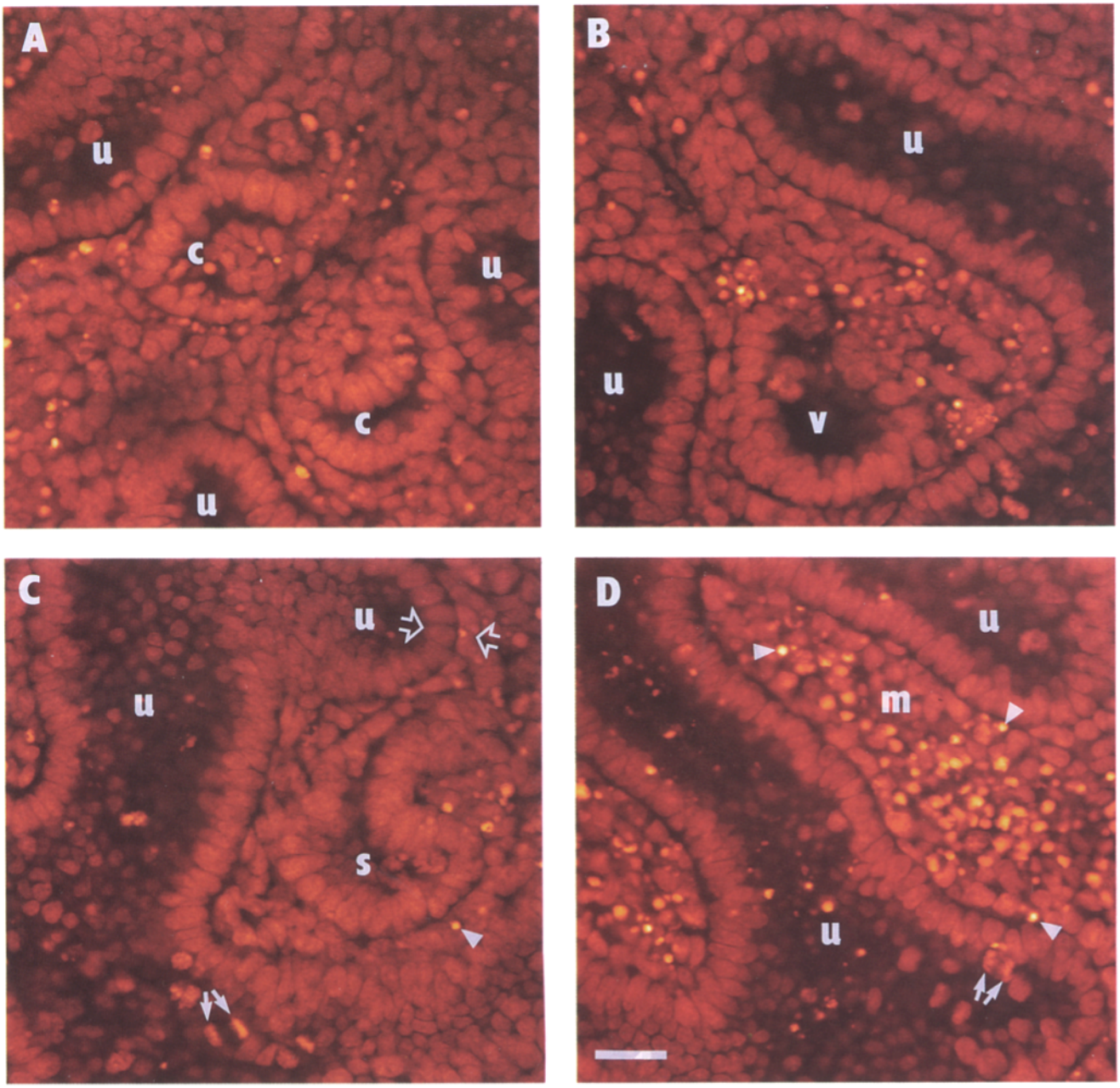

Figure 10. Tissue effects of anti-HGF/SF antibody. E11 metanephric rudiments were cultured for 48 h in the presence of basal serum-free medium with control antibody (*A* and *C*) or with 10 mg/l anti-HGF/SF IgG Fab_2 fragments (*B* and *D*), and they were visualized by confocal microscopy after staining with propidium iodide. *A* and *B* represent outer areas of the organ in the vicinity of the tips of the ureteric bud branches, while *C* and *D* show inner areas of the explants. In organs grown with control antibody, S-shaped bodies (*s*) and comma-shaped nephron precursors (*c*) have formed from mesenchyme (*m*) between the branches of the ureteric bud (*u*): in *C*, the open arrows indicate the close proximity of the distal end of an S-shaped body with a branch tip of the ureteric bud. In the presence of the anti-HGF/SF antibody, no normal nephron precursors formed in the inner part of the organ (*D*), although occasional deformed vesicle-like structures (*v*) were noted in the outer parts (*B*). Apoptotic nuclei appear irregular, small and bright (*arrowheads*) and mitotic nuclei are indicated by closed arrows. All fields contain apoptotic nuclei, and the anti-HGF/SF antibody significantly increased apoptosis in mesenchyme in the center of the organ (*D*). Bar, 25 μm.

Figure 9. Blockade of HGF/SF in metanephric organ culture. Stereomicroscope images of E11 (*A–C*) and E12 (*F–K*) rudiments after 3 d of culture in serum-free basal media alone (*A*, *F*, and *I*), basal media and nonimmune rabbit IgG Fab_2 (*B*, *G* and *J*), and basal media with rabbit anti-mouse HGF/SF IgG Fab_2 (*C*, *H*, and *K*). In *I–K*, E12 organs that had been cultured for 72 h were stained with X-gal to reveal endogenous galactosidase activity, a marker of mature epithelia derived from both the the urtereric bud and renal mesenchyme. Note that ureteric bud branching and nephron formation are limited in organs treated with anti-HGF/SF antibody. Photomicrographs of 10-μm paraffin sections stained with haematoxylin and eosin are shown for an E11 rudiment grown for 3 d in basal medium (*D*) and for the same period in the presence of anti-HGF/SF antibodies (*E*). Note the condensations of mesenchyme around the tips of the ureteric bud in *D* compared with the cystic appearance of the bud surrounded by loose mesenchyme with pyknotic nuclei in *E*. Arrowheads indicate the tips of the ureteric bud and arrows indicate either mesenchymal condensates (*D*) or nephrons (*F* and *I*). *u*, ureteric bud or its derivatives; *m*, mesenchyme. Bars, 100 μm.

Table I. Effects of Anti-HGF/SF Antibodies on Apoptosis in E11 Metanephros Cultured for 48 h

	Whole organ (n = 98 fields)	Peripheral mesenchyme (n = 46 fields)	Medulla of metanephros (mesenchyme and ureteric epithelia) (n = 46 fields)
Basal medium	7.44 ± 0.70	6.02 ± 0.76	8.58 ± 1.10
HGF/SF (100 pM)	9.35 ± 0.89	7.40 ± 1.10	11.10 ± 1.31
Control Ab	9.24 ± 0.77	8.46 ± 1.09	10.12 ± 1.06
Anti-HGF/SF Ab	*12.89 ± 1.01	9.05 ± 1.12	*15.64 ± 1.47

Results are expressed as apoptotic nuclei per high power field as mean ± SEM
*$P < 0.01$ vs control antibody and also vs basal medium alone. n = 8 organs in each group.

by E11 and E12 explants was inhibited when anti-HGF/SF antibodies were added to the organ culture (Figs. 9 and 10). Furthermore, on the basis that the addition of antibodies to HGF/SF was associated with morphological evidence of cell death in the renal mesenchyme, we suggest that HGF/SF may act as an endogenously produced cell survival factor in the renal mesenchyme.

Expression of Met in E11 Mesenchyme

The results of the RT-PCR analysis (Fig. 2), in situ hybridization (Fig. 3), and antibody staining (Fig. 4) on E11 organs suggested that both *HGF/SF* and *met* genes are expressed in the E11 primitive renal mesenchyme. In a previous study (Sonnenberg et al., 1993), it was reported that met mRNA expression was confined to renal epithelia or their immediate precursors, but the E11 renal mesenchyme contains no nephron epithelia, nor their immediate precursors, the comma and S-shaped bodies, which do not appear until E13 (Fig. 1). The resolution of histological techniques, however, can not resolve gene expression at the level of single cells. Therefore, to clarify the phenotypes of individual mesenchymal cells with regard to HGF/SF and met, we generated conditionally immortal cell lines from the primitive renal mesenchyme using mice carrying a temperature-sensitive SV-40 T Ag transgene (Jat et al., 1991). These cells were derived from the primitive mesenchyme and not from the ureteric bud for three reasons: (*a*) the ureteric bud was removed in the original dissection; (*b*) we have consistently failed to establish cell lines from the isolated ureteric bud using the same transgenic model (Woolf, A. S., unpublished results); and (*c*) none of the cell lines expressed cytokeratin, a marker of E11 ureteric bud epithelium (Lehtonen et al., 1985). The pattern of met expression in early nephrogenesis described in our study is consistent with the immunochemical data in whole organs recently reported by Tsarfaty et al. (1994).

Of 13 clonal lines that we established from the E11 renal mesenchyme, all had either the HGF/SF$^+$/met$^+$ or the HGF/SF$^-$/met$^+$ phenotype. They stained for syndecan and vimentin, consistent with identities as renal mesenchymal cells that had received inductive signals from the ureteric bud (Vainio et al., 1989). One line (A1, HGF/SF$^-$/met$^+$) expressed uvomorulin, a cell adhesion molecule characteristic of condensing renal mesenchyme in vivo, and it formed a cobblestone appearance in monolayer culture. Clonality of the line was subsequently confirmed by demonstrating a single integration site of a neotransducing retrovirus (Woolf, A. S., M. Kolatsi, E. Gherardi, E. Andermarcher, L. G. Fine, P. S. Jat, and M. D. Noble. 1993. *J. Am. Soc. Nephrol.* 4:480 [Abstr.]). On the basis of these characteristics, we suggest that this line represents a cell that has entered a mesenchymal to epithelial transition. This hypothesis is supported by preliminary data in which we found that this line formed compact aggregates that resembled condensates after transplantation into the renal cortex of neonatal mice (Woolf, A. S., M. Kolatsi, E. Gherardi, E. Andermarcher, L. G. Fine, P. S. Jat, and M. D. Noble. 1993. *J. Am. Soc. Nephrol.* 4:480 [Abstr.]), a milieu that supports the growth of transplanted metanephric tissue (Woolf et al., 1990). A second clonal line (M5, HGF/SF$^+$/met$^+$) did not express uvomorulin and appeared to have a more fibroblastic phenotype in monolayer culture, suggesting that it was less differentiated than the A1 line. Recently, Karp et al. (1994) reported the isolation of a cell line from a later stage of mouse nephrogenesis (E13.5). The line had some features of an undifferentiated renal mesenchymal cell and, although these cells expressed met, HGF/SF expression was not investigated (Karp et al., 1994).

Although Tsarfaty et al. (1994) have suggested that expression of met in HGF/SF$^+$ fibroblasts may initiate a program of epithelial differentiation, our results with the conditionally immortal cell lines suggest a different conclusion. HGF/SF$^+$/met$^+$ lines, such as clone M5, did not have an epithelial phenotype in monolayer culture. Conversely, the HGF/SF$^-$/met$^+$ phenotype of the A1 line was associated with characteristics to be expected of precursors of nephron epithelia, including the expression of uvomorulin. Thus, during kidney organogenesis, expression of met in cells expressing HGF/SF is not sufficient to induce the appearance of epithelial markers. The reason why cell lines expressing both HGF/SF and met (such as clone M5) did not proliferate in response to exogenous HGF/SF is not clear. It is worth noting, however, that we have obtained similar results with certain 3T3 fibroblast lines that express both HGF/SF and met (Moorby, C., and E. Gherardi, unpublished results).

Effects of Anti-HGF/SF Antibody and HGF/SF in Metanephric Organ Culture

The anti-HGF/SF antibody produced three effects in organ cultures of metanephros: it inhibited the differentiation of metanephric mesenchymal cells into the epithelial precursors of the nephron, it increased apoptosis within the renal mesenchyme, and it perturbed branching morphogenesis of the ureteric bud. These effects were not observed in cultures containing FBS (not shown), suggesting that other cytokines in serum can substitute for HGF/SF, and they are distinct from those that result from exposure to antibody to the laminin A chain (Klein et al., 1988). The latter treatment did not perturb branching of the ureteric bud or produce mesenchymal death, but instead it prevented lumen formation by condensed renal mesenchyme. Although other antisera (e.g., to IGFs and TGF-α [Rogers et al., 1991 and 1992] also perturb nephrogenesis in organ culture, the histology in those experiments were not reported in much detail, so comparison of those experiments with the current study are difficult to make. A recent short report by Santos et al. (1994) produced preliminary evidence that antibodies to HGF/SF could perturb nephrogenesis, but the specific tissue effects were not reported, and the cellular source and biological targets of metanephric HGF/SF were not investigated in detail.

We found that exogenous HGF/SF slightly, but significantly, increased mitosis within the peripheral mesenchyme, but did not alter morphology compared to rudiments grown in serum free media. The lesser effects of exogenous HGF/SF compared to immunological blockade of HGF/SF suggests that the endogenously produced factor is producing a near maximal biological effect during early nephrogenesis.

Putative Roles of HGF/SF in Kidney Development

As discussed in the introduction, it has been suggested that a number of molecules, including several cytokines, may be involved in nephrogenesis. Genetic analysis in transgenic mice carrying targeted null mutations, however, has established that relatively few genes appear essential for normal nephrogenesis in vivo. These include *WT-1*, a gene encoding a transcription factor expressed in the renal mesenchyme (Kreidberg et al., 1993) and the *ret* protooncogene that is expressed in the ureteric bud (Schuchardt et al., 1994). The in vivo roles of other genes are less clear. For example, mice lacking a functional IGF II gene (De Chiara et al., 1990) develop apparently normal kidneys, even though a role for this molecule in nephron formation and branching of the ureteric bud had been proposed from experiments with metanephric organ cultures (Rogers et al., 1991). Similarly, mice with null mutations of the gene for the low affinity nerve growth factor receptor develop normal kidneys (Lee et al., 1992), while the disruption of expression of the same gene in organ culture has produced specific aberrations of nephrogenesis in the hands of some investigators (Sariola et al., 1991) but not others (Durbeej et al., 1993).

On the grounds of the pattern of expression of the *HGF/SF* and c-*met* genes during nephrogenesis and the effects of the anti-HGF/SF antibody on organ cultures, we propose that HGF/SF and met may play a role both in nephron formation and the branching of the ureteric bud. Our experiments in organ culture, however, can not demonstrate that HGF/SF has a direct activity on these processes because of the complex inductive interactions between the ureteric bud and renal mesenchyme. Future studies will be required to assess the effects of the factor when these tissues are cultured in isolation (Perantoni et al., 1991*a*, *b*). More important, the potential activity of HGF/SF during nephrogenesis remains to be investigated in vivo.

Epithelial branching morphogenesis occurs during the development of the lung, pancreas, thymus, liver, prostate, salivary, and mammary glands, and it requires the presence of mesenchymal cells or mesenchymally derived factors (Grobstein, 1967; Bard and Ross, 1991). This observation suggests that there exist molecules derived from the mesenchyme that regulate epithelial growth and differentiation. HGF/SF may be one such molecule based on its pattern of expression (Sonnenberg et al., 1993) and the functional data shown in Figs. 9 and 10. Another molecule that may enhance epithelial morphogenesis is epimorphin, a cell surface protein expressed in a wide range of mesenchymes (Hirai et al., 1992). On the other hand, the wide distribution of TGF-β together with its inhibitory effects on morphogenesis (Daniel et al., 1989; Rogers et al., 1993; Hardman et al., 1994*b*) suggests that TGF-β may terminate epithelial morphogenesis. Intriguingly, TGF-β has been reported to inhibit the secretion of HGF/SF by adult fibroblasts (Gohda et al., 1992) and also to antagonize HGF/SF-induced epithelial branching in cell culture (Santos and Nigam, 1993). These results indicate that expression of these molecules may be important in the initiation and termination of epithelial morphogenesis in several organs, including the kidney.

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References

Bard, J. B. H. 1990. Morphogenesis. The Cellular and Molecular Processes of Developmental Anatomy. Cambridge University Press, Cambridge. 291 pp.

Bard, J. B. H., and A. S. A. Ross. 1991. LIF, the ES-cell inhibition factor, reversibly blocks nephrogenesis in cultured mouse kidney rudiments. *Development (Camb.).* 113:193–198.

Birchmeier, C., and W. Birchmeier. 1993. Molecular aspects of mesenchymal-epithelial interactions. *Annu. Rev. Cell Biol.* 9:511–540.

Bottaro, D. P., J. S. Rubin, D. L. Faletto, A. M.-L. Chan, T. E. Kmiecik, G. F. Vande Woude, and S. A. Aaronson. 1991. Identification of the hepatocyte growth factor as the c-*met* proto-oncogene product. *Science (Wash. DC).* 251:802–804.

Chan, A. M.-L., H. W. S. King, E. A. Deakin, P. R. Tempest, J. Hilkens, V. Kroezen, D. R. Edwards, A. J. Wills, P. Brookes, and C. S. Cooper. 1988. Characterization of the mouse *met* protooncogene. *Oncogene.* 2: 593–599.

Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid-guanidinium-thiocyantate-phenol-chloroform extraction. *Anal. Biochem.* 162:156–159.

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

Daniel, C. W., G. B. Silberstein, K. Van Horn, P. Strickland, and S. Robinson. 1989. TGF-β1 induced inhibition of mouse mammary ductal growth: developmental specificity and characterisation. *Dev. Biol.* 135:20–30.

De Chiara, T. M., A. Efstratiadis, and E. J. Robertson. 1990. A growth-deficiency phenotype in heterozygous mice carrying and insulin-like growth factor II gene disrupted by targeting. *Nature (Lond.).* 345:78–80.

Durbeej, M., S. Soderstrom, T. Ebendal, C. Birchmeier, and P. Ekblom. 1993. Differential expression of neurotropin receptors during renal development. *Development (Camb.).* 119:977–986.

Furlong, R. A., T. Takheara, W. G. Taylor, T. Nakamura, and J. S. Rubin. 1991. Comparison of biological and immunochemical properties indicates that scatter factor and hepatocyte growth factor are indistinguishable. *J. Cell Sci.* 100:173–177.

Gherardi, E., J. Gray, M. Stoker, M. Perryman, and R. Furlong. 1989. Purification of scatter factor, a fibroblast-derived basic protein that modulates epithelial interactions and movement. *Proc. Soc. Natl. Acad. Sci. USA.* 86:5844–5848.

Gohda, E., T. Matsunaga, H. Kataoka, and T. Yamamoto. 1992. TGF-β is a potent inhibitor of hepatocyte growth factor secretion by human fibroblasts. *Cell Biol. Int. Reports.* 16:917–926.

Grobstein, C. 1967. Mechanisms of organotypic tissue interactions. *Natl. Cancer Inst. Monogr.* 26:279–299.

Hardman P., B. J. Klement, and B. S. Spooner. 1993. Growth and morphogenesis of embryonic mouse organs on Biopore membrane. *In Vitro Cell Dev. Biol.* 26:1119–1120.

Hardman, P., M. Kolatsi, P. J. Winyard, P. R. Towers, and A. S. Woolf. 1994*a*. Branching out with the ureteric bud. *Exp. Nephrol.* 2:211–219.

Hardman, P., E. Landels, A. S. Woolf, and B. S. Spooner. 1994*b*. Transforming growth factor-β1 inhibits growth and branching morphogenesis in embryonic mouse submandibular and sublingual glands. *Dev. Growth Diff.* In press.

Harlow, E., L. V. Crawford, D. C. Pim, and N. M. Williamson. 1981. Monoclonal antibodies specific for Simian virus tumour antigens. *J. Virol.* 39: 861–869.

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

Hirai, Y., K. Takebe, M. Takashine, S. Kobayashi, and M. Takeichi. 1992. Epimorphin: a mesenchymal protein essential for epithelial morphogenesis.

Cell. 69:385–387.

Jat, P. S., M. D. Noble, P. Ataliotis, Y. Tanaka, N. Yannoutsos, L. Larsen, and D. Kioussis. 1991. Direct derivation of conditionally immortal cell lines from an H-2K^b-tsA58 transgenic mouse. *Proc. Natl. Acad. Sci. USA.* 88: 5096–5100.

Karp, S. L., A. Ortiz-Arduan, S. Li, and E. G. Neilson. 1994. Epithelial differentiation of metanephric mesenchymal cells after stimulation with hepatocyte growth factor or embryonic spinal cord. *Proc. Natl. Acad. Sci. USA.* 91:5286–5290.

Klein, G., M. Langegger, R. Timpl, and P. Ekblom. 1988. Role of laminin A chain in the development of epithelial cell polarity. *Cell.* 55:331–341.

Koseki, C., D. Herzlinger, and Q. Al-Awqati. 1992. Apoptosis in metanephric development. *J. Cell Biol.* 119:1322–1333.

Kreidberg, J. A., H. Sariola, J. M. Loring, M. Maeda, J. Pelletier, D. Housman, and R. Jaenisch. 1993. WT-1 is required for early kidney development. *Cell.* 74:679–691.

Lee, K.-F., E. Li, J. Huber, S. C. Landis, A. H. Sharpe, M. V. Chao, and R. Jaenisch. 1992. Targeted mutation of the gene encoding the low affinity NGF receptor p75 leads to deficits in the peripheral sensory nervous system. *Cell.* 69:737–749.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 277:680–685.

Lehtonen, E., I. Virtanen, and L. Saxen. 1985. Reorganisation of intermediate filament cytoskeleton in induced metanephric mesenchyme cells is independent of tubule morphogenesis. *Dev. Biol.* 108:481–490.

Matsudaira, P. 1987. Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. *J. Biol. Chem.* 262: 10035–10038.

Montesano, R., K. Matsumoto, T. Nakamura, and L. Orci. 1991. Identification of fibroblast-derived epithelial morphogen as hepatocyte growth factor. *Cell.* 67:901–908.

Nakamura, T., T. Nishizawa, M. Hagiya, T. Seki, M. Shimonishi, A. Sugimura, K. Tashiro, and S. Shimizu. 1989. Molecular cloning and expression of human hepatocyte growth factor. *Nature (Lond.).* 342:440–443.

Naldini, L., K. M. Weidner, E. Vigna, G. Gaudino, A. Bardelli, C. Ponzetto, R. P. Narsimhan, G. Hartmann, R. Zarnegar, and G. K. Michalopoulos. 1991. Scatter factor and hepatocyte growth factor are indistinguishable ligands for the MET receptor. *EMBO (Eur. Mol. Biol. Organ.) J.* 10: 2867–2878.

Perantoni, A. O., L. F. Dove, and C. L. Williams. 1991*a*. Induction of tubules in rat metanephrogenic mesenchyme in the absence of an inductive tissue. *Differentiation.* 48:25–31.

Perantoni, A. O., C. L. Williams, and A. L. Lewellyn. 1991*b*. Growth and branching morphogenesis of rat collecting duct anlagen in the absence of metanephrogenic mesenchyme. *Differentiation.* 48:107–113.

Platt, J. L., D. M. Brown, K. Granlund, T. R. Oegema, and D. J. Klein. 1987. Proteoglycan metabolism associated with mouse metanephric development: morphologic and biochemical effects of β-D-xyloside. *Dev. Biol.* 123: 293–306.

Ponzetto, C., A. Bardelli, Z. Zhen, F. Maina, P. dalla-Zonca, S. Giordano, A. Graziani, G. Panayotou, and P. M. Comoglio. 1994. A multifunctional docking site mediates signalling and transformation by the hepatocyte growth factor/scatter factor receptor family. *Cell.* 77:261–271.

Rogers, S. A., G. Ryan, and M. R. Hammerman. 1991. Insulin-like growth factors I and II are produced in the metanephros and are required for growth and development in vitro. *J. Cell Biol.* 113:1447–1453.

Rogers, S. A., G. Ryan, and M. R. Hammerman. 1992. Metanephric transforming growth factor-α is required for renal organogenesis in vitro. *Am. J. Physiol.* 262:F533–F539.

Rogers, S. A., G. Ryan, A. F. Purchio, and M. R. Hammerman. 1993. Metanephric transforming growth factor-β1 regulates nephrogenesis in vitro. *Am. J. Physiol.* 264:F996–F1002.

Rothenpieler, U. W., and G. R. Dressler. 1993. *Pax-2* is required for mesenchyme-to-epithelium conversion during kidney development. *Development (Camb.).* 119:711–720.

Santos, O. F. P., E. J. G. Barros, X.-M. Yang, K. Matsumoto, T. Nakamura, M. Park, and S. K. Nigam. 1994. Involvement of hepatocyte growth factor in kidney development. *Dev. Biol.* 163:525–529.

Santos, O. F. P., and S. K. Nigam. 1993. HGF-induced tubulogenesis and branching of epithelial cells is modulated by extracellular matrix and TGF-β. *Dev. Biol.* 160:293–302.

Sariola, H., E. Aufderheide, H. Bernhard, B. Henke-Fahle, W. Dippold, and P. Ekblom. 1988. Antibodies to cell surface ganglioside GD3 perturb inductive epithelial mesenchymal interactions. *Cell.* 54:235–245.

Sariola, H., M. Saarma, K. Sainio, U. Arumae, J. Palgi, A. Vaatokari, I. Thesleff, and A. Karavanov. 1991. Dependence of kidney morphogenesis on the expression of nerve growth factor receptor. *Science (Wash. DC).* 254:571–573.

Saunders, S., M. Jalkanen, S. O'Farrell, and M. Bernfield. 1989. Molecular cloning of syndecan, an integral membrane proteoglycan. *J. Cell Biol.* 108:1547–1556.

Saxen, L. 1987. Organogenesis of the Kidney. Cambridge University Press, Cambridge, UK. 340 pp.

Schuchardt, A., V. D'Agati, L. Larsson-Blomberg, F. Costantini, and V. Pachnis. 1994. Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret. *Nature (Lond.).* 367:380–383.

Sonnenberg, E., D. Meyer, K. M. Weidner, and C. Birchmeier. 1993. Scatter factor/hepatocyte growth factor and its receptor, the c-met tyrosine kinase, can mediate a signal exchange between mesenchyme and epithelia during mouse development. *J. Cell Biol.* 123:223–235.

Stoker, M., and M. Perryman. 1985. An epithelial scatter factor released by embryo fibroblasts. *J. Cell Sci.* 77:209–223.

Stoker, M., E. Gherardi, M. Perryman, and J. Gray. 1987. Scatter factor is a fibroblast-derived modulator of epithelial cell mobility. *Nature (Lond.).* 327:239–242.

Tashiro, K., M. Hagiya, T. Nishizawa, T. Seki, M. Shimonishi, S. Shimizu, and T. Nakamura. 1990. Deduced primary structure of rat hepatocyte growth factor and expression of the mRNA in rat tissues. *Proc. Natl. Acad. Sci. USA.* 87:3200–3204.

Tsarfaty, I., S. Rong, J. H. Resau, S. Rulong, P. P. da Silva, and G. F. Vande Woude. 1994. The met proto-oncogene mesenchymal to epithelial cell conversion. *Science (Wash. DC).* 263:98–101.

Vainio, S., M. Jalkanen, M. Bernfield, and L. Saxen. 1989. Transient expression of syndecan in mesenchymal cell aggregates of the embryonic kidney. *Dev. Biol.* 152:221–232.

Vestweber, D., and R. Kemler. 1985. Identification of a putative cell adhesion domain of uvomorulin. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:3393–3398.

Weller, A., L. Sorokin, E.-M. Illegen, and P. Ekblom. 1991. Development and growth of mouse embryonic kidney in organ culture and modulation of development by soluble growth factor. *Dev. Biol.* 144:248–261.

Weidner, K. M., M. Sachs, and W. Birchmeier. 1993. The Met receptor tyrosine kinase transduces motility, proliferation and morphogenetic signals of scatter factor/hepatocyte growth factor in epithelial cells. *J. Cell Biol.* 121:145–154.

Whitehead, R. H., P. E. VanEeden, M. D. Noble, P. Ataliotis, and P. S. Jat. 1993. Establishment of conditionally immortalised epithelial cell lines from both the colon and small intestine of adult H-2K^b-tsA58 transgenic mice. *Proc. Natl. Acad. Sci. USA.* 90:587–591.

Wilkinson, D. G., J. A. Bailes, and A. P. McMahon. 1987. A molecular analysis of mouse development from 8 to 10 days post coitum detects changes only in embryonic globin expression. *Development (Camb.).* 99:493–500.

Woolf, A. S., S. J. Palmer, M. J. Snow, and L. G. Fine. 1990. Creation of a functioning chimeric mammalian kidney. *Kidney Int.* 38:991–997.