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Induction of nephrogenic mesenchyme by osteogenic protein 1 (bone morphogenetic protein 7)

(transforming growth factor type β superfamily/epithelial–mesenchymal interaction/kidney development)

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ABSTRACT **The definitive mammalian kidney forms as the result of reciprocal interactions between the ureteric bud epithelium and metanephric mesenchyme. As osteogenic protein 1 (OP-1/bone morphogenetic protein 7), a member of the TGF-β superfamily of proteins, is expressed predominantly in the kidney, we examined its involvement during metanephric induction and kidney differentiation. We found that OP-1 mRNA is expressed in the ureteric bud epithelium before mesenchymal condensation and is subsequently seen in the condensing mesenchyme and during glomerulogenesis. Mouse kidney metanephric rudiments cultured without ureteric bud epithelium failed to undergo mesenchymal condensation and further epithelialization, while exogenously added recombinant OP-1 was able to substitute for ureteric bud epithelium in restoring the induction of metanephric mesenchyme. This OP-1-induced nephrogenic mesenchyme differentiation follows a developmental pattern similar to that observed in the presence of the spinal cord, a metanephric inducer. Blocking OP-1 activity using either neutralizing antibodies or antisense oligonucleotides in mouse embryonic day 11.5 mesenchyme, cultured in the presence of metanephric inducers or in intact embryonic day 11.5 kidney rudiment, greatly reduced metanephric differentiation. These results demonstrate that OP-1 is required for metanephric mesenchyme differentiation and plays a functional role during kidney development.**

The metanephros arises when the ureteric bud grows into the primitive nephrogenic mesoderm and induces the formation of metanephric tubules (1–4). The cells that are destined to convert into nephrons aggregate around the branching tips of the ureteric bud to undergo condensations characterized by the expression of specific markers, such as WT-1, Wnt-4, Pax-2, uvomorulin (E-cadherin), and laminin (5–9). The process of metanephric induction can be prevented *in vivo* by interruption of nephric duct ingrowth and can be modeled *in vitro*, where the inducing tissue can be homologous (ureteric bud) or heterologous (spinal cord or salivary gland).

The signal that induces *in vitro* tubulogenesis has not yet been fully characterized. A variety of molecules (for review, see ref. 10) participate in metanephric differentiation *in vitro*, including transferrin, GD3 ganglioside, and laminin (9). In addition, several growth factors and their receptors influence metanephric differentiation, including transforming growth factor (TGF)-α and -β (11, 12), basic fibroblast growth factor (13), insulin-like growth factor I and II (14), insulin-like growth factor I receptor (15), nerve growth factor receptor (16), epidermal growth factor (12) and activin (17), hepatocyte growth factor and its presumptive receptor, the *met* gene product (18). Recently, Wnt-1 spatial expression has been shown to be capable of initiating condensation and tubulogenesis in uninduced mesenchyme (19). Although several growth factors such as insulin-like growth factor I and insulin-like growth factor II (20, 21) and hepatocyte growth factor (22) were shown to stimulate metanephric development *in vitro*, the kidneys appeared to be normal in the null mutation mice. On the other hand, mice lacking WT-1 (23), Wnt-4 (6), or Pax-2 (24), all manifest renal aplasia, suggesting that these molecules are critical in metanephric development.

Bone morphogenetic proteins (BMPs)/osteogenic proteins (OPs) are growth and differentiation factors and are members of the TGF-β superfamily. These proteins were identified based on their ability to induce the formation of endochondral bone at extraskeletal sites (for review, see ref. 25). Several BMPs/OPs are also present at sites of epithelial–mesenchymal interaction during mammalian development (26–29). The kidney has been identified as the major site for synthesis of OP-1 during human and mouse development (30) and BMP-7/OP-1 null mutation mice manifest several renal dysplasia (31–32).

In the present study, we investigated the temporal and spatial distribution of OP-1 RNA during early embryonic kidney development and the role of OP-1 in metanephric differentiation *in vitro*. These developmental and functional studies provide evidence that OP-1 mediates mesenchymal–epithelial signaling that are required for normal metanephric development.

MATERIALS AND METHODS

Mouse Tissue and Metanephric Culture. Metanephric culture was performed according to standard techniques (1, 3, 4). In some metanephric rudiments obtained at embryonic day (E)11.5, the ureteric bud was removed by manipulation in 0.02% EDTA. For later stages, the intact E11.5 metanephric rudiment was cultured. Metanephric tissue was placed on Nucleopore filters (Nucleopore, Pleasanton, CA); in some cases, fragments of inducing tissue (spinal cord) were included below the filter. Chemically defined medium consisted of Iscove's modified Eagle's medium (IMEM)-ZO (GIBCO/BRL), supplemented with 50 μg of human transferrin per ml (Collaborative Research) and 4 mM glutamine (GIBCO/BRL). For OP-1 blocking experiments using phosphorothioate oligonucleotides, we compared IMEM-ZO with MEM. Serum medium was composed of IMEM-ZO or MEM supplemented with 10% fetal calf serum (GIBCO/BRL), 50 μg of transferrin per ml, and 4 mM glutamine. The kidneys were scored on the basis of 1–5, 6–10, and >10 glomeruli and/or ureter bud branches.

RNA Studies. Northern hybridization and *in situ* hybridization were performed as described (26, 28).

Antisense Phosphorothioate Oligonucleotide Experiments. Isoform-specific portions of the murine OP-1 have been selected

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Abbreviations: TGF, transforming growth factor; BMP, bone morphogenetic protein; OP, osteogenic protein; E, embryonic day.

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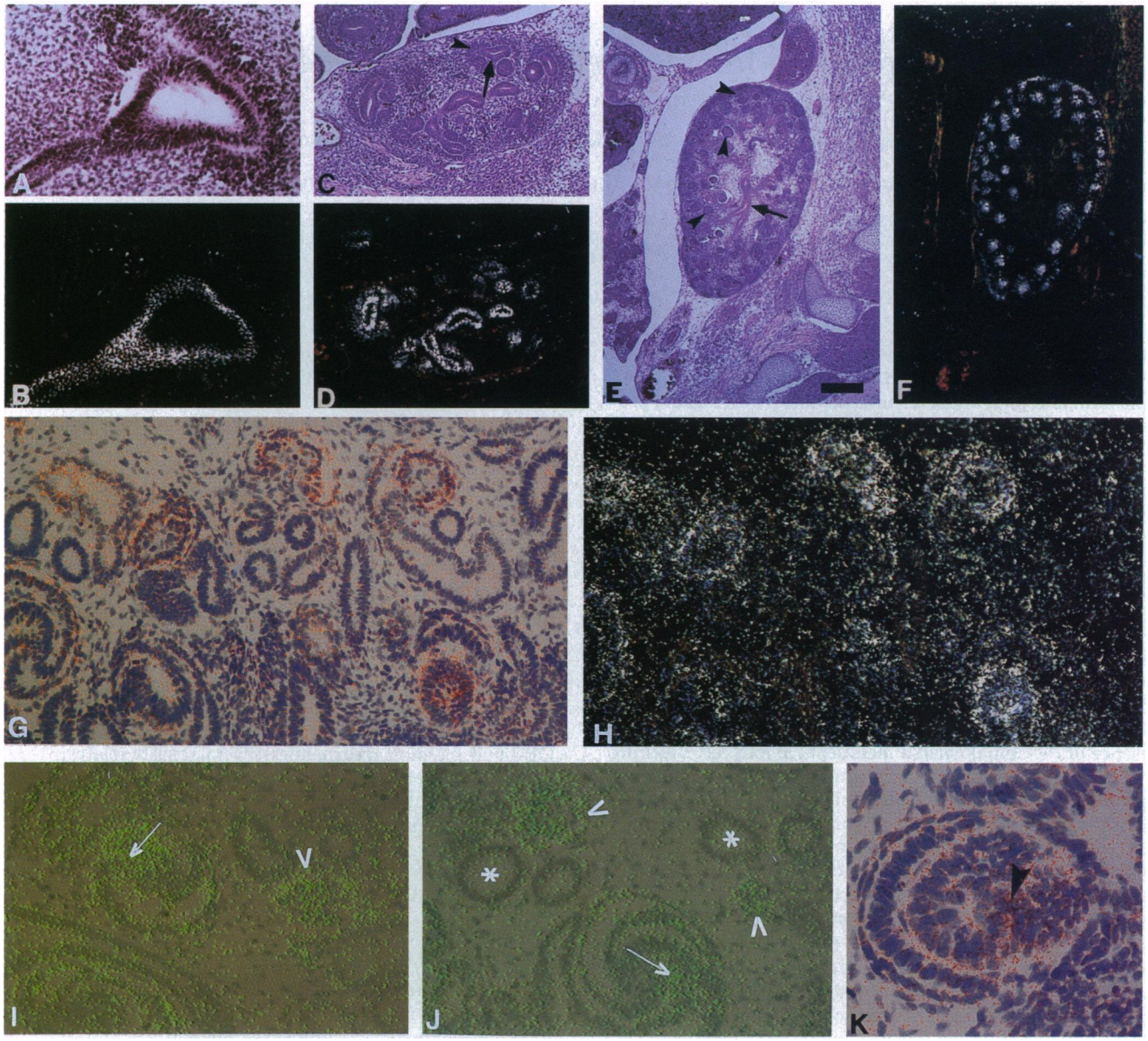

FIG. 1. Localization of OP-1 mRNA during mouse kidney development by *in situ* hybridization. In E11.5 embryonic metanephros, OP-1 transcripts are present in the epithelium of the ingrowing ureteric bud, but absent from mesenchyme (*A* and *B*). At E12.5, OP-1 transcripts are present both in ureteric bud epithelium and induced mesenchymal condensates adjacent (*C*, arrowhead) to the ureteric bud (*C* and *D*, arrow). At E14.5, OP-1 transcripts are present in mesenchymal condensates (*I* and *J*, arrowheads), comma (*I*, arrow), and S-shaped (*J*, arrow) bodies and in vascularized glomeruli (*G* and *H*, arrowheads; *E* and *K*), and they remain absent in tubules derived from the ureteric bud (*E* and *F*, arrow). No OP-1 transcripts were detected in convoluted tubules and collecting ducts (*G* and *H*; *J*, asterisk). *A*, *C*, and *E*, brightfield; *B*, *D*, *F*, and *H*, darkfield; and *G*, *I*, *J*, and *K*, epipolarization. (*E*, bar = 200 μm.)

as follows: AS-1 OP-1 (antisense), 5′-TGGTGGTATCGAGGGTGGT; S-1 OP-1 (sense), 5′-TCCACCCTCGATACCACCA; AS-2 OP-1 (antisense), 5′-GACCGGATACTACGGAGAT; S-2 OP-1 (sense), 5-ATCTCCGTAGTATCCGGTC; AS-3 OP-1 (antisense), 5′-TGCCTCTGGTCACTGCTGC; S-3 OP-1 (sense), 5′-GCAGCAGTGACCAGAGGCA; and NS-3 OP-1 (nonsense), 5′-CGACTAGAGCGCGAACGAC.

The AS-1 and S-1 sequences correspond to nucleotides in the pro region of OP-1; AS-2 and S-2 sequences correspond to nucleotides in the RXXR region of the pro OP-1 maturation site. AS-3 and S-3 OP-1 covers nucleotides just before the first cysteine of the mature region. Phosphorothioate oligonucleotides were prepared on a DNA synthesizer (Millipore) and purified using HPLC. Control oligonucleotides include sense and nonsense oligonucleotides, which were selected to contain a similar base composition that lacks sequences complementary to other mammalian mRNAs.

Recombinant OP-1, cDNA, and Antibodies. Recombinant OP-1 was used in either a mature or soluble form (33), and the concentration was expressed as mature equivalent. An irrelevant recombinant protein (herpes simplex virus glycoprotein D) was used in control experiments. Probes specific for *WT-1* (5), *Pax-2* (gift from H. Westphal, National Institute of Child Health and Human Development; ref. 7), *Wnt-4* (gift from A. McMahon, Harvard University; ref. 6), and *laminin α-1* (gift from Y. Yamada, National Institute on Dental Research) were used as described. The following antibodies were used: Pax-2 (a gift from G. R. Dressler, University of Michigan), uvomorulin (Clone Decma-1, Sigma), EHS laminin (a gift from H. K. Kleinman, National Institute on Dental Research), and OP-1 (1B12-D3 and 12G3; ref. 27).

RESULTS

OP-1 Gene Expression Correlates Temporally with Epithelial–Mesenchymal Interactions During Initiation of Kidney

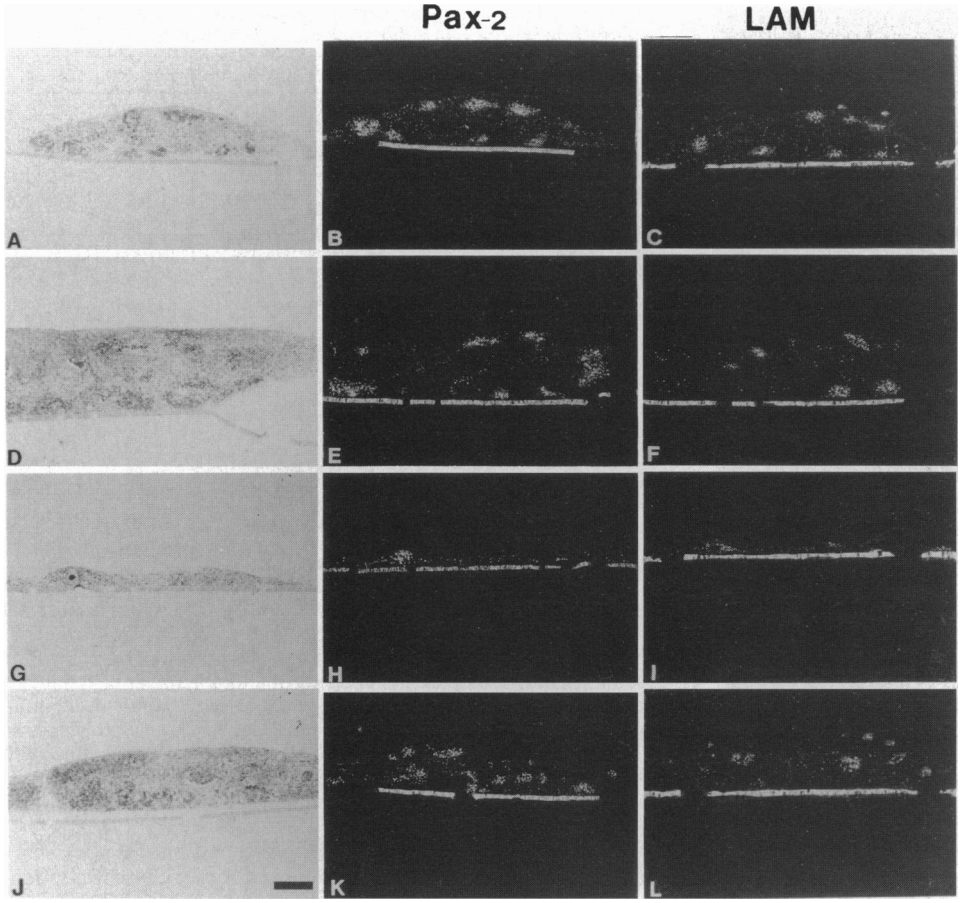

FIG. 2. OP-1 induces tubulogenesis of isolated mouse E11.5 metanephric mesenchyme and promotes expression of specific metanephric differentiation marker gene. Mouse E11.5 nephrogenic mesenchyme was cultured for 54 h in transfilter culture. In *A–C*, metanephric culture in the absence of spinal cord and the presence of 100 ng of OP-1 per ml was associated with mesenchymal condensation and tubulogenesis (*A*), with *Pax-2* (*B*), and *laminin α-1* RNA (*C*) expression. In *D–F*, metanephric culture in the presence of spinal cord and 2.5 μM sense oligonucleotide (S-3) showed similar tubulogenesis and marker gene expression. In *G–I*, metanephric culture in the presence of spinal cord and 2.5 μM antisense oligonucleotide (AS-3) showed limited tubulogenesis (G) and little or no *Pax-2* (*H*) and *laminin α-1* RNA expression. In *J–L*, metanephric culture in the presence of spinal cord and 2.5 μM nonsense oligonucleotide exhibited normal metanephric development. Sections in *A*, *D*, *G*, and *J* were stained with toluidine blue; other sections were photographed in darkfield. The size differences among the sections reflect the size of tissue sectioning. (×100. *J*, bar = 50 μm.)

Development. Fig. 1 shows that fetal mouse kidney is a major site of OP-1 mRNA synthesis. The OP-1 transcripts were first detected in the epithelium of the ureteric bud outgrowing from the nephrogenic cord at E11.5 (Fig. 1 *A* and *B*). At E12.5, OP-1 gene expression persisted within the ureteric bud and was also present in the mesenchymal condensates surrounding the ureteric bud (Fig. 1 *C* and *D*). At E14.5, OP-1 expression was exclusively within the mesenchymal cell aggregates and developing tubules (Fig. 1 *E*, *F*, *I*, and *J*). Each aggregate undergoes epithelialization and proceeds through comma- and S-shaped developmental stages, which are the precursors of the glomeruli and renal tubules, before differentiating into a nephron. During the morphogenesis of comma- and S-shaped shaped bodies, OP-1 mRNA was expressed in the epithelial cells (Fig. 1 *I* and *J*), while in more mature glomeruli OP-1 mRNA was detected also in cells of the ingrown capillaries (Fig. 1 *G*, *H*, and *K*). No OP-1 transcripts were detected in proximal and distal tubules, ureteric bud derivatives, or the interstitium (Fig. 1 *G–J*). Cellular immunostaining colocalizes with distribution of RNA (ref. 27 and data not shown).

OP-1 Promotes Cell Condensations and Tubulogenesis in E11.5 Metanephric Mesenchyme. Metanephric inducers like ureteric bud (Fig. 1) and spinal cord (data not shown) express OP-1 mRNA, as determined by *in situ* hybridization. Evaluation of the effect of OP-1 on cultured renal mesenchyme from E11.5 mouse embryos show that while mouse kidney metanephric rudiment cultured without ureteric bud failed to undergo tubulogenesis, exogenously added recombinant OP-1 was able to induce the metanephric mesenchyme to undergo condensation and tubulogenesis (Figs. 2 and 3; Table 1). Nephrogenesis induced by OP-1 follows a similar developmental pattern as evident with other metanephric inducers as shown by the expression of Wnt-4 (data not shown), Pax-2, uvomorulin/E-cadherin, and laminin α-1, all markers that are characteristic of nephrogenic development. BMP-2, a closely related member of the TGF-β superfamily, and TGF-β1 had no effect on metanephric differentiation under identical conditions (data not shown).

Blocking studies show that when E11.5 nephrogenic mesenchyme was grown in the presence of an inducer (spinal cord), addition of OP-1 antisense oligonucleotides inhibited the induction of the mesenchymal condensation and subsequent tubulogenesis, as followed by the expression of specific marker genes (Fig. 2 and Table 1). Addition of 2.5 μM antisense, sense, or nonsense OP-1 oligonucleotide had no effect on *WT-1* mRNA expression in metanephric mesenchyme with or without an inducer or with exogenous recombinant OP-1 (data not shown). On the other hand, *Pax-2* mRNA was absent from uninduced mesenchyme but appeared when metanephric rudiments were cultured with spinal cord or added OP-1. OP-1 antisense oligonucleotide inhibited *Pax-2* expression, while OP-1 sense oligonucleotide had no effect. Patterns of expression similar to *Pax-2* mRNA were also observed for *Wnt-4* mRNA (data not shown) and *laminin α-1* mRNA using the same culture conditions (Fig. 2).

OP-1 Autoregulates Its Expression During Metanephric Differentiation. Analysis of the E11.5 metanephric explants cultured in the presence of ureteric bud for 3 days showed a high level of OP-1 gene expression in the mesenchymal cells adjacent to the epithelium (Fig. 3 *C* and *D*). However, when explants were cultured without ureteric bud, there was no OP-1 mRNA expression observed (Fig. 3 *A* and *B*). These data indicate that OP-1 expression in mesenchyme is dependent on epithelial–mesenchymal interactions during budding of the ureter into the condensing mesenchyme. The addition of 5–10 ng of OP-1 per ml to the nephrogenic mesenchyme cultured in the absence of ureteric bud epithelium turned on OP-1 mRNA expression within 48–72 h in the condensing cells (Fig. 3*F*), while TGF-β1 and/or BMP-had no effect on OP-1 mRNA expression (data not shown). Blocking studies using OP-1

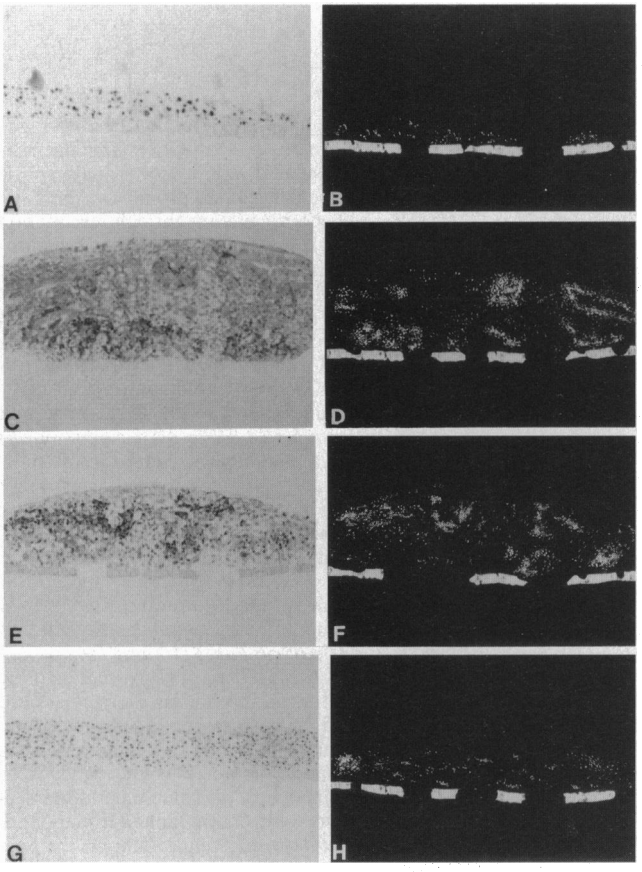

FIG. 3. OP-1 RNA expression in isolated E11.5 metanephros is upregulated by OP-1 and by inducing tissues. Mouse E11.5 nephrogenic mesenchyme was separated from ureteric bud and cultured for 52 h in the presence of the following: medium alone (*A* and *B*), ureteric bud epithelium placed adjacent to metanephros (*C* and *D*), soluble OP-1 (*E* and *F*), and spinal cord placed adjacent to metanephros together with OP-1 specific monoclonal antibody (*G* and *H*). Expression of OP-1 RNA transcripts was detected by *in situ* hybridization. In the absence of inducers, metanephric tissue shows little or no differentiation with small and dense nuclei (*A*) and no OP-1 transcripts (*B*). Ureteric bud induced cell condensations and tubulogenesis (*C*) and OP-1 transcripts within metanephros, localized to mesenchymal aggregates and tubules (*D*). The addition of 10 ng of OP-1 per ml had a similar effect (*E* and *F*). Spinal cord worked equally well as an inducing tissue (data not shown), and 5 μg of specific anti-OP-1 monoclonal antibody per ml inhibited metanephric induction by spinal cord, as shown by a low level of OP-1 expression compared with culture in the absence of any inducing tissue (*G* and *H*). *A*, *C*, *E*, and *G* were photographed in brightfield and *B*, *D*, *F*, and *H* were photographed in darkfield, all at ×100. (*H*, bar = 50 μm.)

antibodies (Fig. 3 *G* and *H*) show a remarkable reduction in OP-1 message in the condensing mesenchyme, suggesting that OP-1 protein secreted by the spinal cord was required for regulation of OP-1 mRNA expression in the responding metanephric mesenchyme.

OP-1 Regulates Metanephric Differentiation in Mouse Intact Kidney Rudiments. Evaluation of the effect of OP-1 on E11.5 intact kidney rudiment cultures showed an increase in tissue differentiation with the formation of more mesenchymal condensations, advanced tubular structures and glomeruli, increased staining for Pax-2 and uvomorulin (Fig. 4 *A*, *B*, *E*, and *F*; Table 2). The growth, as determined by DNA and protein content, was comparable to both OP-1 treated and untreated control (data not shown), while BMP-2 and TGF-β1 reduced the growth in E11.5 kidney cultures (data not shown). In kidney rudiments from E12.5 and older embryos, which express OP-1 mRNA endogenously, the addition of exogenous

Table 1. Temporal expression of mesenchymal and epithelial markers in mouse E11.5 nephrogenic mesenchyme cultures

	Expression of markers			
Treatment/markers	24–35 h	36–48 h	49–72 h	73–96 h
Spinal cord				
Pax-2	13/14	18/18	15/19	2/16
Wnt-4	15/17	22/22	11/19	1/20
laminin α-1	2/19	20/20	10/10	12/12
Uvomorulin	2/15	21/22	20/20	24/24
OP-1				
Pax-2	10/12	20/22	16/19	4/17
Wnt-4	13/16	19/22	10/20	2/18
laminin α-1	1/16	18/21	19/19	14/15
Uvomorulin	2/17	16/20	21/21	23/24
Spinal cord plus OP-1 AS-3 oligo				
Pax-2	2/16	2/17	1/14	0/16
Wnt-4	3/17	2/18	1/14	0/16
laminin α-1	0/13	5/24	3/20	3/20
Uvomorulin	0/11	4/23	3/21	2/21

Metanephric mesenchyme was isolated free of ureteric bud and cultured as described, with spinal cord or OP-1 (10 ng per ml) or spinal cord plus OP-1 antisense oligonucleotide AS-3 (2.5 μM). The data represent, for each duration of culture, the number of metanephric rudiments expressing each marker for the total number of rudiments examined. Treatment with OP-1 resulted in metanephric differentiation comparable to culture with spinal cord, while OP-1 antisense oligonucleotide blocked metanephric differentiation in culture with spinal cord. Treatment with recombinant human BMP-2 (10 ng per ml) induced no structures in 15 metanephroi (data not shown).

OP-1 (20 ng per ml) reduced proliferation as assessed by DNA content and increased the number of mesenchymal condensates (data not shown). These results suggest that OP-1 may influence mesenchymal cell condensation and subsequent tubulogenesis, perhaps by governing local OP-1 expression during kidney morphogenesis.

Intact kidney rudiments treated with sense or nonsense OP-1 oligonucleotides exhibited a normal growth and differentiation pattern comparable to control cultures, whereas antisense oligonucleotide at a concentrations of 2.5 μM inhibited mesenchymal cell condensations and the formation of glomeruli (Fig. 4 *C*, *D*, *G*, and *H*; Table 2). Histological evaluation showed that the mesenchyme was loosely organized with few or no comma- or S-shaped bodies present, and limited ureteric bud branching was observed. Similar effects were found using specific OP-1 neutralizing monoclonal antibodies 1B12 or 12G3 (Fig. 4 *I* and *J*). The blocking of OP-1 activity by OP-1 antibodies or AS-3 oligonucleotide does not appear due to premature cell death or altered cell fate commitment, as determined by the viability score (data not shown). Control experiments showed that AS-3 oligonucleotide inhibited OP-1 mRNA in cultured kidney rudiments but had no effect on BMP-3 expression (Fig. 5). In the presence of sense and nonsense oligonucleotides, the OP-1 mRNA expression levels were maintained (Fig. 5). The effect of antisense oligonucleotides was comparable when the metanephric cultures were maintained in IMEM and MEM containing oligonucleotides at concentrations of up to 2.5 μM.

DISCUSSION

The present study demonstrates that OP-1/BMP-7 mediates an early inductive signal for metanephric differentiation during the development of vertebrate kidney. The evidence is based on the following findings: (*i*) OP-1 is expressed in the ureteric bud epithelium before mesenchymal condensation at E11 in mouse embryo; (*ii*) recombinant human OP-1 substitutes for the ureteric bud epithelium in mouse E11.5 nephrogenic mesenchyme cultures and initiates cell condensation and

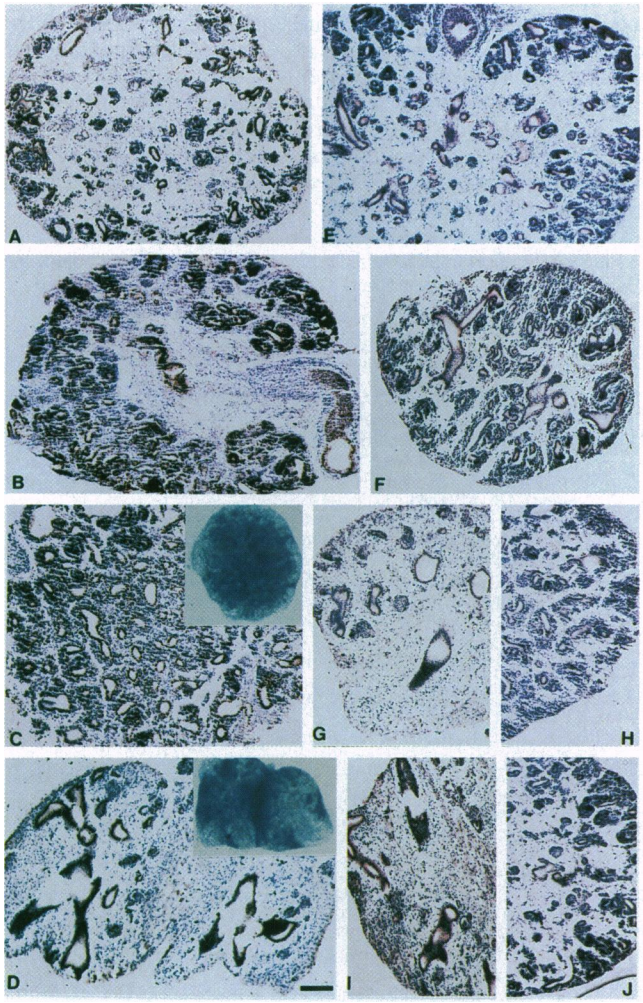

FIG. 4. Expression of differentiation marker proteins in E11.5 metanephric rudiments treated with OP-l and OP-1 inhibitors. Mouse E11.5 metanephric rudiments, isolated with the ureteric bud left in place to allow induction to occur, were cultured for 72 h together with OP-1 or OP-1 inhibitors, and differentiation marker proteins Pax-2 and uvomorulin were detected by immunohistochemistry. With medium alone, mesenchymal condensations and tubular structures stained for Pax-2 (*A*) and tubules were stained for the epithelial marker uvomorulin (*E*). Culture in the presence of 20 ng of OP-1 per ml increased the number of structures expressing Pax-2 (*B*) and uvomorulin (*F*). Culture in the presence of 2.5 μM OP-1 sense oligonucleotide S-3 had no effect on the expression of Pax-2 protein (*C*, with *Inset*) or uvomorulin (*H*). By contrast, culture in the presence of 2.5 μM OP-1 antisense oligonucleotide AS-3 reduced the number of structures expressing Pax-2 (*D*, with *Inset*) and uvomorulin (*G*). Similarly, culture in the presence of 5 μg of OP-1 specific monoclonal antibody per ml inhibited uvomorulin expression (*I*) while 5 μg of the irrelevant herpes simplex specific monoclonal antibody per ml had no effect. (Tissue sections were photographed in brightfield at a magnification of ×100, except for the metanephric rudiments photographed in phase contrast at ×25 in the *Insets* of *C* and *D*. *D*, bar = 200 μm.)

subsequent tubulogenesis; and (*iii*) the blocking of OP-1 activity using either neutralizing antibodies or antisense oligonucleotides in the kidney rudiment explant cultures inhibits the developmental cascade of cellular events, leading to metanephric differentiation. These observations collectively indicate that OP-1 is required for kidney development and comprise the required embryological criteria for an inducing morphogenic signal (34). The finding that OP-1 is expressed in other known metanephric inducers including spinal cord and salivary gland (28) further supports the role of OP-1 for metanephric differentiation.

Table 2. Effects of OP-1 and OP-1 inhibitors on branching morphogenesis and development of glomeruli in mouse E11.5 intact kidney rudiments cultured for 72 h

Treatment	Concentration	Total no.	Branching morphogenesis			Glomeruli		
			1-5	6-10	>10	1-5	6-10	>10
Control	—	38	0	3	35	0	3	35
OP-1	5 ng/ml	51	0	6	45	0	5	46
OP-1	50 ng/ml	32	0	2	30	0	1	31
AS-3 oligo	2.5 μM	26	11	12	3	18	8	0
S-3 oligo	2.5 μM	20	0	2	18	0	1	19
NS-3 oligo	2.5 μM	18	0	3	15	0	1	17
1B12 antibody	5 μg/ml	24	12	11	1	17	7	0
HS antibody	5 μg/ml	14	0	1	13	0	1	13

Mouse E11.5 metanephric mesenchyme with ureteric bud left intact was cultured for 72 h in chemically defined medium as described, with the following factors: medium alone, OP-1, OP-1 antisense oligonucleotide AS-3, OP-1 sense oligonucleotide S-3, nonsense oligonucleotide NS-3, OP-1-specific monoclonal antibody (1B12) and herpes simplex specific antibody (HS). The number of branching tubules and glomeruli was counted, using both phase contrast and hematoxylin- and eosin-stained serial sections.

The OP-1 expression observed in the condensing mesenchyme and during tubulogenesis is induced by metanephric inducers and dependent on the presence of OP-1, suggesting the possibility of OP-1 autoregulation during kidney development. Analysis of upstream regulatory sequences of OP-1 gene revealed the presence of *Egr-1* and *WT-1* binding sequences (E. Ozkaynak, personal communication). These zinc-finger proteins may possibly regulate OP-1 expression during early stages of metanephric differentiation. The absence of *WT-1* may result in impaired OP-1 mRNA expression in condensing mesenchyme, and *WT-1*-governed OP-1 gene expression *in situ* may be necessary for proper mesenchymal cell condensation and subsequent epithelialization. The findings that OP-1 expression precedes *Wnt-4* and *Pax-2* expression and that blocking the OP-1 activity inhibits their expression suggest that OP-1 mediates an early step involved in nephrogenesis. The gene knockout studies have shown that *WT-1*, *Wnt-4*, and *Pax-2* genes are required for proper kidney development.

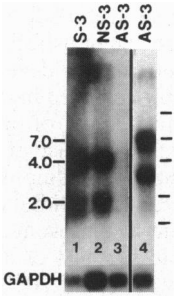

FIG. 5. Northern analysis of OP-1 expression in metanephric rudiments: specific inhibition by OP-1 antisense oligonucleotides. Mouse E11.5 kidney rudiments with the ureteric bud left in place were cultured for 96 h as described; sense oligonucleotide (S-3, lane 1), nonsense oligonucleotide (NS-3, lane 2), and antisense oligonucleotide (AS-3, lane 3) were added daily at a concentration of 2.5 μM. Poly(A)+ RNA was prepared, and Northern analysis was performed using an OP-1-specific cDNA probe (lanes 1 and 3), a BMP-3-specific cDNA probe (lane 4), and a normalizing gene, a glyceraldehyde-6-phosphate dehydrogenase (GAPDH)-specific cDNA probe. The x-ray films were exposed for 6 days to membranes hybridized with OP-1 and BMP-3 probes and for 18 h to the same membrane hybridized with a GAPDH probe. RNA size markers are indicated at right (9, 7, 4.2, 2.2, and 1.3 kb). The OP-1 mRNA expression was similar with sense and nonsense oligonucleotides but was substantially reduced following culture with antisense oligonucleotide. Antisense oligonucleotide had no effect on BMP-3 RNA expression.

The use of antisense oligonucleotides in metanephric cultures requires precise documentation of culture conditions, uptake, and suppression of RNA expression (35–38). In the present study, we show that OP-1 AS-3 oligonucleotide specifically inhibits OP-1 expression in both basal medium (MEM) and enriched medium (IMEM) at concentrations up to 2.5 μM. In addition, we found that the oligonucleotides are taken up by mesenchymal cells, while uptake was substantially less by the E11.5 ureteric bud epithelium and was absent from the ureteric bud epithelium at later time points. The ureteric bud epithelium has been previously noted to be refractory to oligonucleotide entry (35–37). Further, we showed that antisense oligonucleotide selectively reduced OP-1 mRNA expression, while sense and nonsense oligonucleotides had no effect. Finally, neutralizing antibodies intended to block the activity of OP-1 present in metanephric cultures prevented differentiation. Thus, reduction of OP-1 mRNA expression and inhibition of OP-1 protein activity both suppressed tubulogenesis in metanephric rudiments.

Two recent reports show that the majority of BMP-7/OP-1 null mutation mice die of renal failure within the first day of postnatal life (31, 32). Luo *et al.* (31) reported the presence of few mesenchymal condensations surrounding ureteric buds at E12.5 and found no glomeruli or tubules at E14.5. The expression of *WT-1*, *Pax-2*, and *Wnt-4* RNA was greatly reduced or nearly absent from the mesenchyme, particularly at E14.5. We suggest that BMP-7/OP-1 is a critical early inducer of nephrogenesis and that placental transfer of maternal BMP-7/OP-1 might partially rescue the embryo and explain the presence of few induced mesenchymal structure. The phenotype reported by Dudley *et al.* (32) was somewhat different, with normal metanephric development up to E14.5 and appropriate expression of *Pax-2*, *Pax-8*, and *Wnt-4*. After this time, mutant kidney exhibited a severely disorganized architecture, few mesenchymal aggregates, and reduced expression of *Pax-2*, *Pax-8*, and *Wnt-4* within the induced mesenchymal structures, while ureteric bud differentiation was preserved. The authors suggest that the absence of BMP-7/OP-1 has little effect on the inductive interaction between ureteric bud and mesenchyme but instead prevents the continued survival and/or differentiation of mesenchymal and epithelial components. Although the reasons for the differences in timing and severity of metanephric defects between these two null mutation mouse models are not readily apparent, these studies clearly demonstrate that BMP-7/OP-1 is required for normal metanephric development.

The organ culture approach used in the present study shows that OP-1 may act as an inducer of undifferentiated mesenchyme, as evidenced by the presence of OP-1 RNA transcripts in various inducing tissues, including uretric bud, and by the ability of recombinant OP-1 to stimulate metanephric development *in vitro*. OP-1 may also prevent apoptosis of uninduced mesenchyme or promote survival of mesenchymal cells that have been induced, as we have used E11.5 metanephric rudiments that have been exposed *in vivo* for several hours to the advancing ureteric bud and may have already received inductive signal(s). Furthermore, OP-1 may function as a differentiation factor, promoting the epithelial conversion of the mesenchyme and/or the continued branching of the ureteric bud, but it may still be dependent upon an inducing factor to initiate the process. Thus, the action of OP-1 in metanephric rudiment system does not allow to unequivocally assign the role of OP-1 as an inductive or survival or differentiation factor. It is likely that OP-1 may govern overall cellular events associated with kidney morphogenesis.

In conclusion, we have shown that OP-1 transcripts are localized *in vivo* to the ureteric bud and subsequently to the induced mesenchyme and that OP-1 is required for and can promote metanephric differentiation *in vitro*. Thus OP-1 plays a critical role in the epithelial–mesenchymal conversion of the metanephros and may serve as a signal for metanephric induction, a survival factor, or enhancer of nephrogenic mesenchyme differentiation. These data suggest a model in which the temporal and spatial expression of OP-1 gene acts in concert with the appropriate responding cell type and permissive microenvironment to confer biological specificity during tissue morphogenesis (39, 40).

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1. Grobstein, C. (1953) *Nature (London)* **172,** 869–871.
2. Grobstein, C. (1955) *J. Exp. Zool.* **169,** 319–340.
3. Saxen, L. (1987) *Organogenesis of the Kidney* (Cambridge Univ. Press, Cambridge, U.K.).
4. Ekblom, P., Miettinen, A., Virtanen, I., Wahlstrom, T., Dawnay, A. & Saxen, L. K. (1981) *Dev. Biol.* **84,** 88–95.
5. Armstrong, J. F., K. Pritchard-Jones, W. A. Bickmore, K. A., Hastie, N. D. & Bard, J. B. L.(1992) *Mech. Dev.* **40,** 85–97.
6. Stark, K., Vainio, S., Vassileva, G. & McMahon, A. P. (1994) *Nature (London)* **372,** 679–683.
7. Dressler, G. R., Deutch, U., Chowdhury, K., Nornes, H. O. & Gruss, P. (1990) *Development (Cambridge, U.K.)* **109,** 787–795.
8. Vestweber, D., Kemmler, R. & Ekblom, P.(1985) *Dev. Biol.* **112,** 213–221.
9. Ekblom, M., Klein, G., Murgrauer, G., Fecker, L., Deutzmann, R., Timpl, R. & Ekblom, P. (1990) *Cell* **60,** 337–346.
10. Bard, J. B. L., McConnell, J. E. & Davies, J. A. (1994) *Mech. Dev.* **48,** 3–11.
11. Avner, E. D. & Sweeney, W. E. J. (1990) *Pediatr. Nephrol.* **4,** 372–377.
12. Weller, A., Sorokin, L., Illgen, E. M. & Ekblom, P. (1991) *Dev. Biol.* **144,** 248–261.
13. Perantoni, A. O., Dove, L. F. & Karavanova, I. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 4696–4700.
14. Rogers, S. A., Ryan, G. & Hammerman, M. R. (1991) *J. Cell Biol.* **113,** 1447–1453.
15. Wada, J., Liu, Z. Z., Alvares, K., Kumar, A., Wallner, E., Makino, H. & Kanwar, Y. S.(1993) *Proc. Natl. Acad. Sci. USA* **90,** 10360–10364.
16. Sariola, H., Saarma, M., Sainio, K., Arumae, U., Palgi, J. V., Vaahtokari, A., Thesleff, I. & Karavanov, A. (1991) *Science* **254,** 571–573.
17. Ritvos, O., Tuuri, T., Eramaa, M., Sainio, K., Hilden, K., Saxen, L. & Gilbert, S. F.(1995) *Mech. Dev.* **50,** 229–245.
18. Woolf, A. S., Kolatsi-Joannou, M., Hardman, P., Andermaher, E., Moorby, C., Fine, L. G., Jat, P. S. M., Noble, M. D. & Cherardi, E. (1995) *J. Cell Biol.* **128,** 171–184.
19. Herzlinger, D., Qiao, J., Cohen, D., Ramakrishna, N. & Brown, A. M. C. (1994) *Dev. Biol* **166,** 815–818.
20. Liu, J.-P., Perkins, A. S., Robertson, E. J. & Efstratiadis, A. (1993) *Cell* **75,** 59–72.
21. Baker, J., Liu, J.-P., Robertson, E. J. & Efstratiadis, A. (1993) *Cell* **75,** 73–82.
22. Schmidt, C., Bladt, F., Goedecke, S., Brinkmann, V., Zschiesche, W., Sharpe, M., Gherardi, E. & Birchmeier, C. (1995) *Nature (London)* **373,** 699–702.
23. Kreidberg, J. A., Sariola, H., Loring, J. M., Maida, M., Pelletier, J., Housman, D. & Jaenisch, R.(1993) *Cell* **74,** 679–691.
24. Torres M., Gomez-Pardo, E., Dressler, G. R. & Gruss, P. (1995) *Development (Cambridge, U.K.)* **121,** 4057–4065.
25. Sampath, T. K. & Rueger, D. C. (1994) *Comp. Orthop.* **9,** 101–107.
26. Vukicevic, S., Helder, M. N. & Luyten, F. P. (1994) *J. Histochem. Cytochem.* **42,** 869–875.
27. Vukicevic S., Latin, V., Chen, P., Batorsky, R., Reddi, A. H. & Sampath, T. K.(1994) *Biochem. Biophys. Res. Commun.* **198,** 693–700.
28. Helder, M. N., Ozkaynak, E., Sampath, T. K., Luyten, F. P., Latin, V., Oppermann, H. & Vukicevic, S. (1995) *J. Histochem. Cytochem.* **43,** 1035–1043.
29. Lyons, K. L., Hogan, B. L. M. & Robertson, E. J. (1995) *Mech. Dev.* **50,** 71–83.
30. Ozkaynak, E., Schnegelsberg, P. N. J., Oppermann, H. (1991) *Biochem. Biophys. Res. Commun.* **179,** 116–123.
31. Luo, G., Hofmann, C., Bronckers, A. L. J. J., Sohocki, M., Bradley, A. & Karsenty, G. (1995) *Genes Dev.* **9,** 2808–2820.
32. Dudley, A. T., Lyons, K. M. & Robertson, E. J. (1995) *Genes Dev.* **9,** 2795–2807.
33. Jones, W. K., Richmond, E. A., White, K., Sasak, H., Kusmik, W., Smart, J., Oppermann, H., Rueger, D. C. & Tucker, R. F. (1994) *Growth Factors* **11,** 215–225.
34. Slack, J. M. W.(1993) *Mech. Dev.* **41,** 91–107.
35. Sainio, K., Saarma, M., Nonclercq, D., Paulin, L. & Sariola, H. (1994) *Cell. Mol. Neurobiol.* **14,** 439–457.
36. Durbeej, M., Sonderstrom, S., Ebendal, T., Birchmeier, C. & Ekblom, P. (1993) *Development (Cambridge, U.K.)* **119,** 977–989.
37. Rothenpieler, U. W. & Dressler, G. R. (1993) *Nucleic Acids Res.* **21,** 4961–4966.
38. Stein, C. A. & Cheng, Y. C. (1993) *Science* **261,** 1004–1012.
39. Reddi, A. H. (1992) *Curr. Opin. Cell Biol.* **4,** 850–855.
40. Sampath, T. K., Rashka, R. K., Doctor, J. S., Tucker, R. F. & Hoffmann, F. M. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 6004–6009.