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The surface ectoderm is essential for nephric duct formation in intermediate mesoderm

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SUMMARY

The nephric duct is the first epithelial tubule to differentiate from intermediate mesoderm that is essential for all further urogenital development. In this study we identify the domain of intermediate mesoderm that gives rise to the nephric duct and demonstrate that the surface ectoderm is required for its differentiation. Removal of the surface ectoderm resulted in decreased levels of *Sim-1* and *Pax-2* mRNA expression in mesenchymal nephric duct progenitors, and caused inhibition of nephric duct formation and subsequent kidney development. The surface ectoderm expresses BMP-4 and we show that it is required for the maintenance of high-level BMP-4 expression in lateral plate mesoderm. Addition of a BMP-4-coated bead to embryos lacking the surface ectoderm restored normal levels of *Sim-1* and *Pax-2* mRNA expression in nephric duct progenitors, nephric duct formation and the initiation of nephrogenesis. Thus, BMP-4 signaling can substitute for the surface ectoderm in supporting nephric duct morphogenesis. Collectively, these data suggest that inductive interactions between the surface ectoderm, lateral mesoderm and intermediate mesoderm are essential for nephric duct formation and the initiation of urogenital development.

Key words: Intermediate mesoderm, Lateral mesoderm, Nephric duct, BMP-4

INTRODUCTION

During embryonic development, intermediate mesoderm differentiates into tubular epithelial tissues of the kidney and genital system. One of the first mesenchymal-to-epithelial conversions that occurs results in the formation of the nephric duct (Saxen, 1987). The nephric duct differentiates into portions of the male genital system and is required for all further kidney development. It induces surrounding intermediate mesoderm to differentiate into nephrons of the mesonephric kidney, the excretory organ of a majority of vertebrate species and a developmental intermediary excretory organ of birds and mammals (Gruenwald, 1942). In birds and mammals, the nephric duct issues a caudal diverticulum, the ureteric bud. The ureteric bud is essential for metanephric kidney morphogenesis; it grows and branches to form the collecting system and induces surrounding intermediate mesoderm to differentiate into nephrons (Saxen, 1987).

Despite its essential role in urogenital development, the regulation of nephric duct formation early in embryogenesis remains poorly characterized. The earliest known regulator of urogenital development is the transcription factor *Pax-2*; however, *Pax-2* null mice form the nephric duct (Torres et al., 1996). Thus, the regulatory genes and signaling factors that induce intermediate mesoderm to differentiate into the nephric duct early in embryogenesis remain elusive.

In this report we used two independent lineage tracing techniques to identify the domain of intermediate mesoderm that gives rise to the nephric duct in the stage-10 chick embryo. We show that removal of the surface ectoderm overlying the nephric duct primordium inhibits nephric duct formation and subsequent kidney development. Furthermore, we demonstrate that BMP-4 can substitute for the surface ectoderm in supporting nephric duct formation. Collectively these data demonstrate that the surface ectoderm is essential for nephric duct formation and that BMP-4 signalling plays a role in this process.

MATERIALS AND METHODS

Chick embryos

Fertilized White Leghorn chicken eggs were obtained from Truslow Farms (NJ) and incubated at 38°C. Embryos were staged according to Hamburger and Hamilton (1951). For more precise staging, somite number was documented. Somite counts included the first somite that disperses at stage 10.

In situ hybridization

Embryos were fixed with 4% paraformaldehyde and processed for

whole-mount in situ hybridization as described (Henrique et al., 1995). After processing, embryos were embedded in paraffin and 10 μm serial sections prepared. *c-Sim-1*,*c-Pax-2* and *c-Bmp-4* plasmids were kindly provided by C. M. Fan (Pourquié et al., 1996), D. Henrique and P. Brickell (Francis et al., 1994), respectively.

Lineage analysis

Stage 10 embryos were injected in ovo with 1 nl of either DiI (Ruiz i Altaba et al., 1993) or 0.5×10^7-10^8 active virions ml^{-1} SNTZ (Fischman and Mikawa, 1997). Eggs were reincubated for 24 hours before fixation with 4% paraformaldehyde. DiI-injected embryos were examined as whole mounts using epifluorescence optics. For histological analysis, embryos were embedded in paraffin and sectioned after DiI photoconversion (Ruiz i Altaba, 1993) or directly cryosectioned (Stern, 1993) and analyzed by epifluorescence optics. SNTZ-injected embryos were processed for β-galactosidase activity, embedded in paraffin, serially sectioned and then stained with Hematoxylin and Eosin.

Removal of the surface ectoderm and preparation of BMP-4-soaked beads

The surface ectoderm overlying paraxial, intermediate and lateral mesoderm was removed from stage 10-12 embryos using tungsten needles from the level of somite 5 to Hensen's node. Since the surface ectoderm grows back by approximately 8 hours post-surgery, carbon particles were used to mark the anterior and posterior levels of ectoderm removal. Eggs were reincubated for given times and then fixed with 4% paraformaldehyde. Affigel blue beads (Vaahtokari et al., 1996) (Bio-Rad) were soaked in 100 ng ml^{-1} BSA or 100 ng ml^{-1} BSA including 0.3-1 μg ml^{-1} BMP-4 (provided by Genetics Institute). Beads were washed extensively in PBS, and a single bead placed on the exposed intermediate mesoderm from which the surface ectoderm was removed at the approximate axial level that gives rise to the mesonephros. Eggs were reincubated for 24 hours and fixed in 4% paraformaldehyde.

RESULTS

Intermediate mesoderm expresses *c-Sim-1* and *c-Pax-2* mRNAs as it differentiates into the nephric duct

By 55 hours of incubation (stage 16; Hamberger and Hamilton, 1951), the nephric duct has formed in the developing chick embryo (Fig. 1A-D). It is characterized by columnar epithelia that express mRNAs encoding *c-Sim-1*, an orthologue of Drosophila *single minded*, and *c-Pax-2* (Pourquié et al., 1996; Dressler et al., 1990). *c-Pax-2* mRNA was also detected in intermediate mesoderm adjacent to the nephric duct (Fig. 1C,D). This domain of intermediate mesoderm, the nephrogenic intermediate mesoderm, differentiates into nephrons following induction by the nephric duct. The temporal and spatial expression patterns of *c-Pax-2* mRNA in the developing avian urogenital system are identical to those observed in the developing mouse; *Pax-2* mRNA is first expressed in the nephric duct and then in nephrogenic intermediate mesoderm after it has been induced to differentiate (data not shown and Dressler et al., 1990). In vitro studies using *Pax*-2 anti-sense oligonucleotides demonstrate that induction of *Pax-2* mRNA expression in nephrogenic intermediate mesoderm is required for the differentiation of this tissue into nephrons (Rothenpieler and Dressler, 1993). Thus, induction of *c-Pax-2* mRNA expression in nephrogenic intermediate mesoderm adjacent to the nephric duct at stage 16 indicates that it has been induced to differentiate into nephrons. Collectively, these results indicate that by stage 16, urogenital development has begun in the developing chick embryo; the nephric duct has formed and it has induced nephrogenesis.

Since nephric duct epithelia co-express *c-Sim-1* and *c-Pax-2* mRNAs, we examined the expression patterns of these mRNAs in intermediate mesoderm at earlier stages of development to determine if they are expressed by nephric duct progenitors. At stage 14, the nephric duct was observed between the axial levels of somites 15-22, and characterized by *c-Sim-1*, *c-Pax-2*-expressing epithelia (data not shown). However, at earlier stages, the nephric duct was not observed at any axial levels. Although the nephric duct was not present at stage 12, *c-Sim-1* and *c-Pax-2* mRNAs were expressed in a cord of mesenchyme that extends along the length of the embryo with its posterior limit caudal to somite 16 (Fig. 1E-H). At stage 10, *c-Pax-2* mRNA expression was localized to a domain of intermediate mesoderm extending from somites 6-10 (Fig. 1K,L); *c-Sim-1* mRNA was expressed by a dorsally located subpopulation of cells in this domain of *c-Pax-2*-expressing intermediate mesoderm (Fig. 1I,J). These mRNA

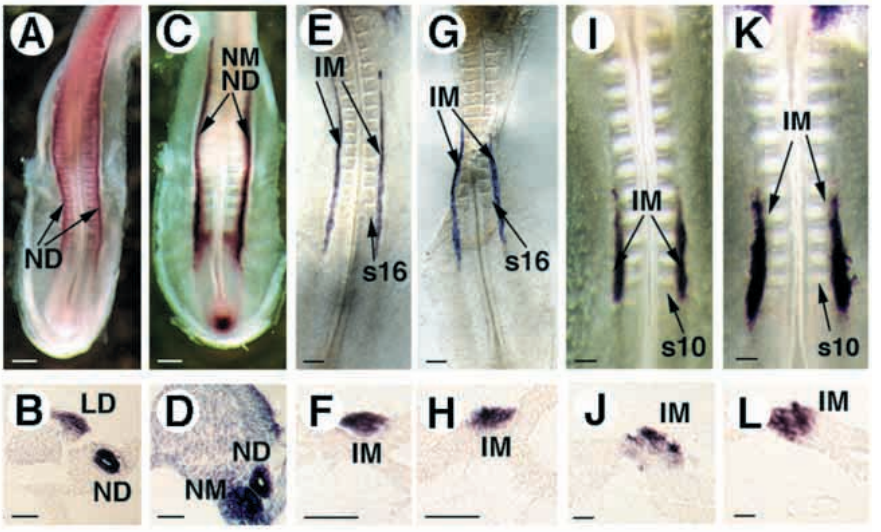

Fig. 1. *c-Sim1* and *c-Pax-2* mRNAs are expressed in intermediate mesoderm as it differentiates into the nephric duct. Whole mount (A,C,E,G,I,K) and paraffin sections (B,D,F,H,J,L) of embryos processed for in situ hybridization detection of *c-Sim-1* (A,B,E,F,I,J) and *c-Pax-2* (C,D,G,H,K,L) mRNAs. At stage 16 (A-D) the nephric duct (ND) is an epithelial tubule that expresses both *c-Sim-1* (A,B) and *c-Pax-2* mRNAs (C,D). *c-Pax-2* mRNA is also expressed in nephrogenic intermediate mesoderm (NM) adjacent to the nephric duct and *c-Sim-1* is expressed in the lateral dermamyotome (LD). At stage 12 (E-H), *c-Sim-1* (E,F) and *c-Pax-2* (G,H) mRNAs are expressed in a cord of intermediate mesoderm (IM) that extends along the embryonic axis with its most posterior limit caudal to somite 16 (s16). At stage 10 (I-L), *c-Sim-1* (I) and *c-Pax-2* (K) mRNA expression is restricted to intermediate mesoderm (IM) between somites 6 and 10 (s10). Examination of sectioned embryos (J,L) demonstrates that *c-Sim-1* mRNA (J) is expressed in a dorsal, subpopulation of *c-Pax-2*-expressing cells (L). Bars, 270 μm (A,C); 100 μm (B,D,E,G,I,K); 25 μm (F,H,J,L).

expression studies, combined with previous experiments in which nephric duct formation was inhibited when intermediate mesoderm between somite levels 9-11 was removed (Le Douarin and Fontain, 1970), raise the possibility that *c-Sim-1*, *c-Pax-2*-expressing intermediate mesoderm at this axial level gives rise to the nephric duct.

To directly confirm this hypothesis, we performed lineage tracing studies using two independent techniques (Fig. 2). Focal domains of intermediate mesoderm (approximately 100 μm^2) along the rostral-caudal embryonic axis of stage 10 chick embryos were injected with DiI or with replication-defective retrovirus encoding *Lac-z* (Ruiz i Altaba et al., 1993; Fischman and Mikawa, 1997). Embryos were analyzed 24 hours after tagging (stage 16). The only domain that gave rise to the nephric duct was the domain of intermediate mesoderm containing cells that coexpressed *c-Sim-1* and *c-Pax-2* mRNAs (n=97; Fig. 2 and data not shown). Furthermore, when the caudal aspect of this domain of intermediate mesoderm adjacent to somite 10 was tagged at stage 10, all nephric duct epithelia were labeled with the lineage marker at stage 16 (Fig. 2c-F). Collectively, these lineage tracing and mRNA expression studies suggest that nephric duct differentiation includes at least two stages: caudal migration of *c-Sim-1*, *c-Pax-2*-expressing mesenchymal nephric duct progenitors, followed by their differentiation into an epithelial tubule that possesses nephron-inducing activity.

The surface ectoderm is required for nephric duct formation

The surface ectoderm lies in close proximity to *c-Sim-1*, *c-Pax-2* mesenchymal nephric duct progenitors (Figs 2B, 3A, 4A) and expresses mRNAs encoding many developmental regulatory signaling molecules including Wnts, Bmps and Fgfs (Parr and McMahon, 1994; Hogan, 1996; Watanabe and Le Douarin, 1996; Yamaguchi and Rossant, 1995; Song et al., 1996). To determine if the surface ectoderm plays a role in the caudal migration or epithelial transformation of nephric duct progenitors, we removed the surface ectoderm from the right side of stage 9+ to stage 12 chick embryos and analyzed them 12-24 hours later. Removal of the surface ectoderm had no effect on nephric duct differentiation in embryos that had 13 or more somites (data not shown). In contrast, nephric duct formation was inhibited when the surface ectoderm was removed from 8- to 12-somite chick embryos (Fig. 3). On the control, unoperated side, the nephric duct was characterized by a columnar epithelium that expressed *c-Pax-2* and *c-Sim-1* mRNAs (Fig. 3B-D). On the operated side of the embryo, a morphologically identifiable nephric duct was absent (Fig. 3B,E,F). However, low levels of *c-Sim-1* and *Pax-2* mRNAs were detected in a cord of mesenchyme in the region of the embryo in which the nephric duct is normally located (Fig. 3E,F). These mRNA expression patterns suggested that mesenchymal nephric duct progenitors migrated caudally but did not undergo tubulogenesis in the absence of the surface ectoderm. Lineage tracing experiments confirm this hypothesis (Fig. 3G,H). Thus, the surface ectoderm is not required for caudal migration of mesenchymal nephric duct progenitors but is essential for the maintenance of high level *c-Sim-1* and *c-Pax-2* mRNA expression in mesenchymal nephric duct progenitors and their differentiation into an epithelial tubule.

In addition to inhibiting nephric duct formation, surface ectoderm removal also inhibited the normal upregulation of *c-Pax-2* mRNA expression in nephrogenic intermediate mesoderm. On the unoperated side of the embryo, high levels of *c-Pax-2* mRNA were detected in nephrogenic intermediate mesoderm indicating that nephron formation was induced (Fig. 3D). On the operated side of the embryo *c-Pax-2* mRNA was dramatically reduced, suggesting that the induction of nephrogenesis did not occur (Fig. 3F). Similar, faint levels of *c-Pax-2* mRNA were observed when the caudal migration of nephric duct progenitors was blocked as described by Gruenwald (1942) (data not shown). Collectively these data suggest that surface ectoderm is required for nephric duct differentiation and nephric duct-dependent nephrogenesis.

Histological analysis of operated embryos allowed to develop to embryonic day (E) 5 provide further support for the role of surface ectoderm in nephric duct morphogenesis and subsequent kidney development. Both the nephric duct and mesonephric nephrons were absent on the operated side of E5

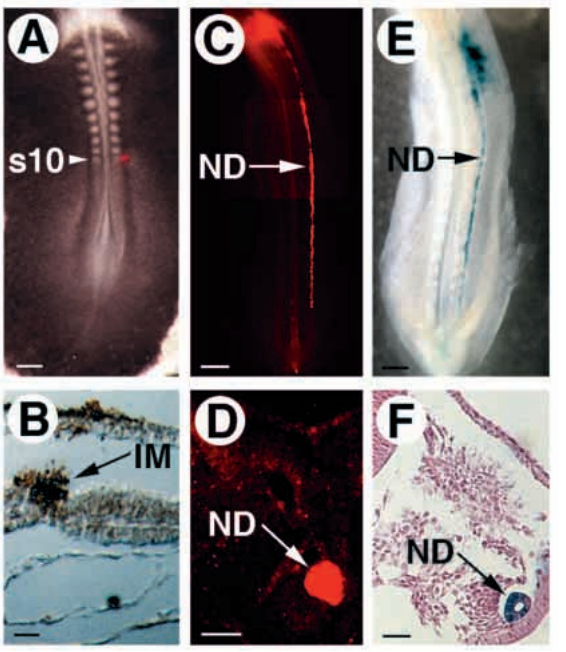

Fig. 2. The nephric duct derives from intermediate mesoderm adjacent to somite 10. Stage-10 embryos were injected in ovo with 1 nl of either DiI, a fluorescent lineage marker or replication-defective retrovirus encoding *lac-z*. Eggs were re-incubated for 24 hours before fixation with 4% paraformaldehyde. (A) Whole-mount image of stage-10 chick embryo after targeted injection of 1 nl of DiI adjacent to somite 10 (s10). (B) 10 μm thick paraffin section through the injection site after DiI photoconversion, demonstrating that intermediate mesoderm (IM) was tagged. (C) Whole-mount image of embryo 24 hours after DiI injection. DiI-labeled cells have migrated caudally and formed the nephric duct (ND). (D) Frozen section from caudal region of embryo injected at stage 10. Fluorescent, DiI-labeled cells are specifically localized within the nephric duct (ND). (E) Whole-mount image of a stage-16 embryo that was injected at stage 10 with 1 nl of SNTZ. *Lac-z*-expressing cells (stained blue) derived from intermediate mesoderm adjacent to somite 10 have migrated caudally and formed the nephric duct (ND). (F) Hematoxylin and Eosin-stained section from caudal region of SNTZ-injected embryo, demonstrating that *Lac-z*-expressing cells are localized specifically to the nephric duct (ND). Bars, 300 μm (A,C,E); 25 μm (B); 90 μm (D,F).

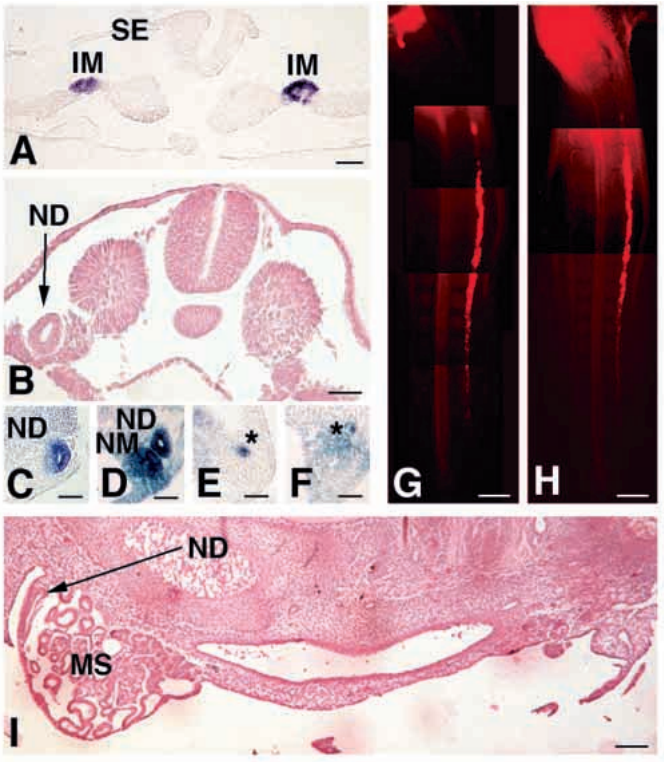

Fig. 3. The surface ectoderm is required for nephric duct formation. The surface ectoderm was removed from the right side of stage-10 embryos. Eggs were reincubated for given times and then fixed with 4% paraformaldehyde. (A) Section of stage-10 embryo fixed immediately after surface ectoderm was removed from the right side and processed for in situ hybridization to detect *c-Sim-1* mRNA. *c-Sim-1*-expressing intermediate mesoderm (IM) appears identical on the operated and unoperated control sides, demonstrating that the nephric duct primordium is not disturbed by surface ectoderm removal. (B) Hemotoxylin and Eosin-stained section of stage-16 embryo 24 hours after the surface ectoderm was removed from the right side. The nephric duct (ND) formed on the control side (left) but was absent on the operated side although surface ectoderm had grown back. On the control side, the nephric duct (ND) was characterized by columnar epithelia that expressed robust levels of both *c-Sim-1* (C) and *c-Pax-2* (D) mRNAs. *c-Pax-2* mRNA was abundantly expressed in nephrogenic mesenchyme (NM). On the operated side, a cord of mesenchyme (*) expressing low levels of *c-Sim-1* and *c-Pax-2* mRNAs (E,F) was present. *c-Pax-2* mRNA was expressed at very low levels in nephrogenic mesenchyme (NM) on the operated side (F). Intermediate mesoderm adjacent to somite 10 was tagged with DiI at stage 10 and the surface ectoderm was left in place (G) or removed (H). Caudal migration of DiI-tagged nephric duct progenitors was assessed 24 hours later by whole-mount fluorescence microscopy. Caudal migration of nephric duct progenitors was identical in control (G) or operated (H) embryos. (I) Hematoxylin and Eosin-stained section of embryo operated at stage 10 and allowed to develop to embryonic day 5. The nephric duct (ND) is surrounded by mesonephric nephrons (MS) on the left, unoperated side while these structures are absent on the side from which surface ectoderm was removed. Bars, 50 μm (A); 90 μm (B-F); 300 μm (G,H); 10 μm (I).

embryos after surface ectoderm was removed from stage 10 embryos (Fig. 3I). Collectively, these data demonstrate that surface ectoderm overlying intermediate mesoderm is required for the initiation of avian urogenital development.

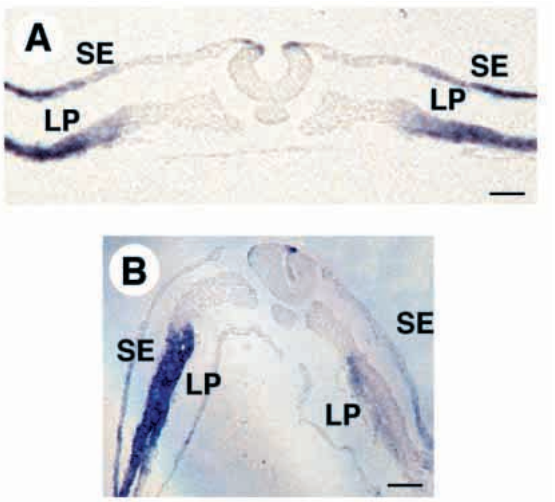

Fig. 4. *c-Bmp-4* mRNA expression levels in close proximity to the nephric duct are regulated by the surface ectoderm. (A) Section of stage-10 embryo caudal to somite 10 processed for in situ hybridization detection of *c-Bmp-4* mRNA. High levels of *c-Bmp-4* mRNA are observed in tissues surrounding the nephric duct as it differentiates, including the surface ectoderm (SE) and lateral plate mesoderm (LP). The surface ectoderm appears displaced from underlying intermediate mesoderm due to processing for paraffin embedding. (B) Section of embryo 8 hours after the surface ectoderm was removed from the right side at stage 10. On the unoperated, control side of the embryo, robust levels of *c-Bmp-4* mRNA are detected in the surface ectoderm (SE) and lateral plate mesoderm (LP). In contrast, on the operated side *c-Bmp-4* expression levels in lateral plate mesoderm and the regenerating surface ectoderm are very low. Bars, 50 μm.

BMP-4 can substitute for the surface ectoderm in inducing nephric duct formation

One of the candidate factors expressed by the surface ectoderm that may be essential for urogenital development is BMP-4 (Hogan, 1996; Watanabe and Le Douarin, 1996). At stages 8 and 9, *c-Bmp-4* mRNA is abundantly expressed by the surface ectoderm (Watanbe and Le Dourain, 1996 and personal observation). By stage 10, high levels of *c-Bmp-4* mRNA are expressed by the surface ectoderm and lateral plate mesoderm (Fig. 4A). Thus, tissues surrounding the nephric duct primordium and nephric duct progenitors, when they transform into an epithelial tubule, express high levels of *c-Bmp-4* mRNA. Strikingly, when the surface ectoderm was removed from 8- to 12-somite embryos, c-*Bmp-4* mRNA expression in lateral plate mesoderm was not maintained (Fig. 4B). Removal of the surface ectoderm from embryos with 13 or more somites did not affect *c-Bmp-4* mRNA expression in lateral plate mesoderm or nephric duct formation (data not shown). Thus, the dependence of nephric duct formation on the surface ectoderm exactly parallels the stages during which the surface ectoderm expresses *c-Bmp-4* mRNA and regulates its transcription in lateral mesoderm.

These data raise the possibility that surface ectoderm-dependent nephric duct formation is mediated by BMP-4 signalling. To directly test this hypothesis, a BMP-4 or control BSA-soaked bead was placed on exposed intermediate mesoderm of stage 10 embryos following removal of the surface ectoderm. Development was assayed at stage 16 (Fig. 5).

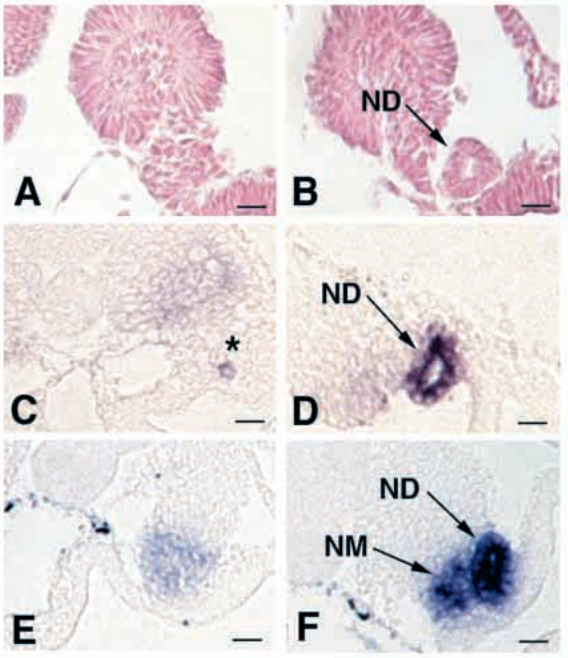

Fig. 5. BMP-4 can substitute for the surface ectoderm in supporting nephric duct formation. Sections of stage-16 embryos 24 hours after a BSA- (A,C,E) or BMP-4- (B,D,F) soaked bead was placed on intermediate mesoderm exposed by removing the surface ectoderm at stage 10. (A) Hematoxylin and Eosin-stained section demonstrates that the nephric duct does not form in the presence of a BSA soaked bead. In contrast, an epithelialized nephric duct (ND) is present when a BMP-4-soaked bead (B) is placed on exposed intermediate mesoderm. *c-Sim-1* mRNA (C,D) can be detected at low levels in a cord of mesenchyme (*) in the presence of a BSA-soaked bead (C) while the BMP-4-soaked bead (D) maintained high levels of *c-Sim-1* mRNA in the nephric duct (ND). *c-Pax-2* mRNA (E,F) was expressed at very low levels in both the nephric duct and nephrogenic mesenchyme of embryos incubated with a BSA-soaked bead (E). In contrast, abundant *c-Pax-2* mRNA expression was present in the nephric duct and adjacent nephrogenic mesenchyme in embryos incubated with a BMP-4-soaked bead (F). Bars, 50 μm

Control BSA-soaked beads did not restore normal *c-Sim-1* or *c-Pax-2* mRNA expression or rescue nephric duct tubulogenesis (n=15; Fig. 5A,C,E). In contrast, a BMP-4 bead induced normal upregulation of both *c-Sim-1* and *c-Pax-2* mRNAs in the nephric duct progenitors and rescued nephric duct tubulogenesis (n=27; Fig. 5B,D,F). Normal levels of *c-Pax-2* mRNA were also observed in nephrogenic intermediate mesoderm adjacent to the rescued nephric duct, indicating that the induction of nephrogenesis had also been restored (Fig. 5F).

DISCUSSION

Although the nephric duct is essential for initiating urogenital development, the tissue interactions and molecules regulating its formation remain elusive. Morphological examination of *c-Sim-1* and *c-Pax-2*-expressing intermediate mesoderm as it differentiates into the nephric duct, combined with lineage tracing experiments, demonstrate that nephric duct progenitors migrate as a cord of mesenchyme along the rostral-caudal embryonic axis and then transform into an epithelial tubule with nephron-inducing activity. Removal of the surface ectoderm from 8- to 12-somite chick embryos inhibits nephric duct differentiation and kidney morphogenesis but has no effect on the caudal migration of mesenchymal nephric duct progenitors. Thus, the surface ectoderm may secrete factors that directly support nephric duct progenitor differentiation. Alternatively, the surface ectoderm may regulate the expression of factors in lateral or paraxial mesoderm that are essential for this process. For example, we show that the surface ectoderm regulates *Bmp-4* mRNA expression levels in lateral mesoderm. Furthermore, when we placed an impermeable barrier between lateral and intermediate mesoderm, nephric duct formation was inhibited (data not shown). Similarly, Mauch et al. (1998) demonstrate that signals from paraxial mesoderm are required for the differentiation of intermediate mesoderm at early stages of urogenital development. Collectively, these data demonstrate that proximate tissue interactions are required for the differentiation of intermediate mesoderm into the nephric duct.

Members of the BMP gene family mediate the differentiation of a variety of cell types. High levels of *c-Bmp-4* mRNA are expressed in both the surface ectoderm and lateral mesoderm, tissues that are in close proximity to the differentiating nephric duct and required for its formation. BMP-4 has been shown to regulate its own transcription (Vainio et al., 1993), and we show that removal of the surface ectoderm dramatically decreases *c-Bmp-4* mRNA expression in lateral mesoderm. Thus, removal of the surface ectoderm dramatically decreases the level of BMP-4 available to mesenchymal nephric duct progenitors as they transform into an epithelial tubule. Furthermore, we show that a BMP-4-coated bead can substitute for the surface ectoderm in supporting nephric duct differentiation. Collectively, these data suggest that the surface ectoderm expresses and regulates the expression of BMP-4, or an unidentified member of the TGF-β family, that is essential for nephric duct differentiation.

Mice haploid for *Bmp-4* exhibit urogenital abnormalities ranging from renal agenesis to cystic kidneys (Dunn et al., 1997). These *Bmp-4* haploinsufficient phenotypes are consistent with abnormal differentiation of intermediate mesoderm at both early and late stages of urogenital development, respectively. Currently, it is not possible to determine if BMP-4 is required for early events in the differentiation of murine intermediate mesoderm because *Bmp-4* haploinsufficient phenotypes are incompletely penetrant and *Bmp-4* null mice die during gastrulation prior to the generation of intermediate mesoderm (Dunn et al., 1997; Winner et al., 1995). Our data demonstrate that BMP-4 levels can modulate *c-Sim-1* and *c-Pax-2* mRNA levels in nephric duct progenitors. Removal of the surface ectoderm, an experimental manipulation that greatly reduces endogenous *c-Bmp-4* mRNA levels, results in a dramatic reduction of *c-Sim-1* and *c-Pax-2* mRNA expression in nephric duct progenitors. Conversely, a BMP-4-coated bead was sufficient to maintain abundant expression of *c-Sim-1 and c-Pax-2* mRNAs in embryos lacking the surface ectoderm. It is unlikely that the maintenance of normal *c-Sim-1* mRNA expression levels in nephric duct progenitors is required for urogenital development since *Sim-1* null mice do not exhibit gross urogenital abnormalities (Jaques et al., 1998). In contrast, *Pax-2* null mice exhibit gross urogenital defects. *Pax-2* null mice form a nephric

duct, but it does not issue the ureteric bud and therefore metanephric kidney development does not occur. Our data suggest that BMP-4 signalling regulates the differentiation of intermediate mesoderm prior to *Pax-2* and is essential for *Pax-2* dependent processes. In conclusion, proximate tissue interactions mediated by BMP-4 or an unidentified member of the TGF-β gene family, are essential for the initiation of urogenital development in intermediate mesoderm.

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