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Patterns of *Evi-1* expression in embryonic and adult tissues suggest that *Evi-1* plays an important regulatory role in mouse development*

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Summary

***Evi-1* is a putative protooncogene first identified as a common site of retroviral integration in murine myeloid leukemias. It encodes a $145\times10^3 M_r$ nuclear DNA-binding protein that contains ten zinc-finger motifs separated into two domains, as well as an acidic domain. These features suggest that *Evi-1* encodes a transcriptional regulatory protein. In *Drosophila*, zinc-finger proteins such as *Kruppel* are involved in body plan patterning, and exhibit a spatially restricted pattern of expression in the embryo. To determine if *Evi-1* may be involved in morphogenetic processes in the mouse embryo, we have performed *in situ* hybridization and Northern blot analysis on embryonic and adult mouse tissues to delineate the spatial and temporal pattern of *Evi-1* expression. Our results show that *Evi-1* is expressed at high levels in a few tissues in the embryo and is widely expressed, albeit at generally low levels, in the adult. Regions that exhibit high-level expression in the embryo include: the urinary system and the Mullerian ducts; the bronchial epithelium of the lung; focal areas within the nasal cavities; the endocardial cushions and truncus swellings in the heart; and the developing limbs. Expression in the limb occurs at the highest levels from 9.5 to 12.5 days, is present in both hind and forelimbs, is absent at the apical ectodermal ridge, and does not appear to establish a gradient. This pattern of expression in the limb is reminiscent of other putative transcriptional factors such as Hox-5.2 and retinoic acid receptor-gamma, consistent with the hypothesis that particular combinations or networks of transcriptional regulatory proteins are required for morphogenesis. Overall, these results suggest that *Evi-1* plays an important role in mouse development.**

Key words: *Evi-1*, zinc-finger protein, mouse embryogenesis, transcription factors, protooncogenes, myeloid leukemogenesis.

Introduction

Investigations into the function of newly identified genes can proceed either by biochemical or genetic approaches. In *Drosophila* and yeast, the ability to create mutations with ease at cloned loci allows one to exploit the strength of genetics to determine gene function. In higher eukaryotes, however, the ability to create mutations in non-selectable genes is now in its infancy, so that the task of defining the function of a gene relies on other approaches, such as describing the spatial and temporal pattern of expression of the gene. Such a description can yield important clues about the role a gene plays throughout development and in the adult animal.

Evi-1 (*e*cotropic *v*iral *i*ntegration site-1) was first identified as a common site of retroviral integration in virally induced myeloid tumors of AKXD mice (Mucenski *et al.* 1988). Subsequent cloning and sequencing of the cDNA corresponding to this locus (Morishita *et al.* 1988) showed that the gene encodes a polypeptide containing ten repeats with extensive homology to the zinc-finger domains first identified in the *Xenopus* transcription factor IIIA (Miller *et al.* 1985) and subsequently found in several other transcriptional regulatory factors. The predicted polypeptide also contains an acidic domain near the carboxy terminus, which is a feature present in several yeast transcription factors and may function as an activation domain for gene transcription (Struhl, 1987). These data suggest that *Evi-1* encodes a DNA-binding protein that acts to regulate mRNA transcription through interaction with one or more specific DNA sequence binding motifs. Indeed, recent studies have shown that Evi-1 is localized to the nucleus (Matsugi *et al.* 1990), and Evi-1 protein binds to DNA (Matsugi *et al.* 1990; Perkins, A. S., R. Fishel, N. A. Jenkins, N. G. Copeland, unpublished data). In myeloid leukemias,

the effect of retroviral insertion is to dramatically increase the transcription of *Evi-1* (Morishita *et al.* 1988), suggesting that increased levels of the protein product contribute to the transformed phenotype. However, the exact role of *Evi-1* in myeloid leukemogenesis and the putative target DNA sequences for Evi-1 binding and action are not known. The myeloid cell lines containing *Evi-1* integrations are dependent on Il-3 for growth and are abnormal in their ability to terminally differentiate (Morishita *et al.* 1988). Thus, it has been proposed that expression of the *Evi-1* gene interferes with normal differentiation of these cells (Morishita *et al.* 1990).

In this report we describe results from Northern and *in situ* hybridization analysis of *Evi-1* expression in adult and embryonic mouse tissues. These data reveal a spatially and temporally restricted pattern of *Evi-1* expression that overlaps that of several other putative transcription factors, such as *Hox-5.2, Hox-5.3* (Dollé and Duboule, 1989; Oliver *et al.* 1989), *Hox-7* (Robert *et al.* 1989; Hill *et al.* 1989) and retinoic acid receptor γ (*RARγ*) (Dollé *et al.* 1989; Ruberte *et al.* 1990), suggesting that *Evi-1* plays a regulatory role during mouse embryogenesis.

Materials and methods

RNA preparation and analysis

Six week old C57BL/6J female mice were killed and trimmed organs were frozen immediately in liquid nitrogen. RNA was prepared as described by Chomczynski and Sacchi (1987). Briefly, tissues were homogenized with a Polytron homogenizer in RNAzol (2 ml per 100 mg tissue), which is a mixture of guanidinium isothiocyanate and phenol (Cinna/Biotecx). The mixture was extracted with chloroform (0.2 ml per 2 ml homogenate), and the aqueous phase was precipitated with an equal volume of isopropanol. Polyadenylated RNA was purified by affinity chromatography using oligothymidilic acid–cellulose (Collaborative Research), as described (Aviv and Leder, 1972). The yield of RNA recovered from the column ranged from 1% to 6%. Fractionation of polyadenylated RNA on agarose gels was performed as described by McMaster and Carmichael (1977) (Fig. 1, left panel), or by Perkins *et al.* (1983) (Fig. 1, right panel). Either 0.5–5 μg (Fig. 1, left panel) or 8–12 μg (Fig. 1, right panel) of RNA were analyzed per lane. RNA was then transferred to nylon membranes (Zetabind; Cuno, Inc.), as described (Thomas, 1980), and hybridized to ^{32}P-labeled probes for *Evi-1*, which were prepared using a Multiprime labeling kit (Amersham). Hybridizations were performed as described by Church and Gilbert (1984) for Fig. 1, left panel or by Wahl *et al.* (1979) for Fig. 1, right panel, except that dextran sulfate was omitted from the prehybridization and hybridization buffers. The filter for Fig. 1, left panel, was washed twice (15 min each) in 0.5 l of buffer A (0.5% bovine serum albumin, 1 mM EDTA, 40 mM phosphate buffer, pH 7.2, and 5% sodium dodecyl sulfate (SDS)) and three times (15 min each) in 0.33 l of buffer B (1 mM EDTA, 40 mM phosphate buffer, pH 7.2, 1% SDS) at 65°C. The filter for Fig. 1, right panel was washed in a final buffer containing 0.1×SSC and 0.1% SDS at 55°C. The filters were then exposed to film for 72 h (Fig. 1, left panel) or 24 h (Fig. 1, right panel) with two intensifying screens at −70°C.

Preparation of probes for in situ *hybridizations*

The 454 bp *Bgl*II–*Hin*dIII fragment of p58.2–1 (Morishita *et al.* 1988), containing the 5′ end of the *Evi-1* cDNA was subcloned into *Bam*HI–*Hin*dIII digested pBluescript KS(−) (Stratagene) using standard techniques. Antisense ^{35}S-labeled *Evi-1* probe was synthesized from this template using T3 polymerase with a RNA Transcription Kit (Stratagene). The probe was purified as described by Sassoon *et al.* (1988). The hybridization probe used for Figs 2H and 3D was labeled with ^{35}S by nick translation as described (Rigby *et al.* 1977) using a 1.2 kb *Sph*I fragment of p58.2–1 which spans a region of *Evi-1* cDNA essentially devoid of zinc fingers.

In situ *hybridizations*

C57BL/6J mice were mated, and pregnant females were killed at various days thereafter. Embryos were dissected, fixed in phosphate-buffered 4% paraformaldehyde, and processed as described (Sassoon *et al.* 1988). For *in situ* hybridization of tissue sections, the technique described by Wilkinson *et al.* (1987), as modified by Sassoon *et al.* (1988) was employed for analysis of embryos. *In situ* hybridizations of adult uterus and kidney (Figs 2H and 3D) were performed as described by Haase *et al.* (1982).

Results

Northern analysis of Evi-1 *expression in adult mouse tissues*

RNA was extracted from several adult mouse organs, poly (A) selected and analyzed on Northern blots using either the full-length *Evi-1* cDNA (Fig. 1, left panel) or a 1.2 kb *Sph*I fragment (bp 1108 to 2365; Morishita *et al.* 1988) that is essentially free of zinc-finger sequences (Fig. 1, right panel) as probes. Neither of these probes shows any cross-hybridization with other loci on Southern blots (data not shown). *Evi-1* expression is found in several adult tissues (Fig. 1). To compare the relative level of *Evi-1* expression in different tissues, we quantitated the relative amount of β-actin transcript in each lane by rehybridizing the Northern blots shown in Fig. 1 with a β-actin probe (Fig. 1, bottom panel). Based on these data, we estimate that high levels of *Evi-1* transcripts are present in ovary and kidney, while lower levels are present in lung, uterus and heart. We have also detected *Evi-1* transcripts in other adult mouse tissues by Northern analysis, including liver, intestine, thymus, spleen, stomach and brain (data not shown).

Two distinct transcripts were detected with the full-length *Evi-1* probe (Fig. 1). A 7.5 kb transcript is predominant in lung, whereas a 6.2 kb RNA is detected in CP1 embryonic stem cells and ovary. Whether the different sized transcripts originate through alternative usage of 5′ transcriptional initiation sites, splicing signals, or polyadenylation signals is not known.

In situ *hybridization for* Evi-1 *expression during mouse embryogenesis*

Evi-1 expression in the mouse embryonic stem cell line, CP1 (Fig. 1), indicates that *Evi-1* is expressed during early embryonic development. To determine the spatial extent of *Evi-1* expression during later time points in

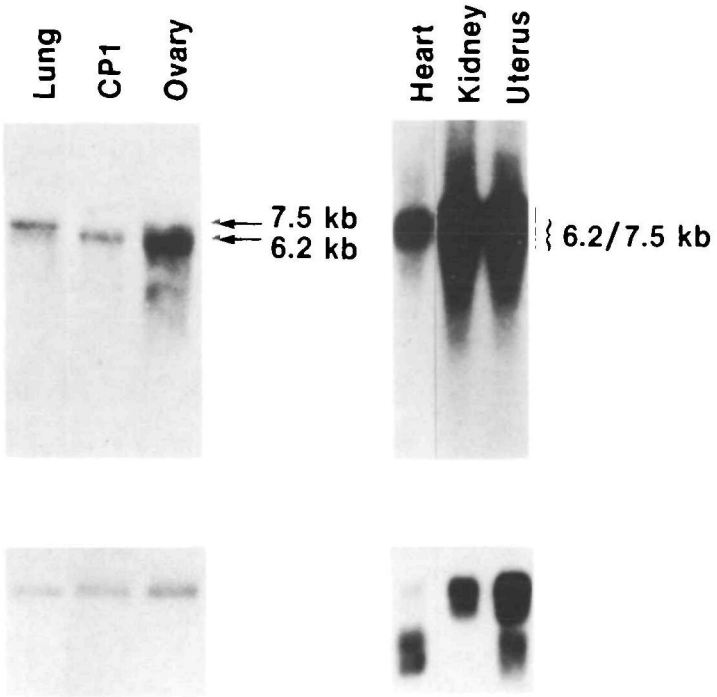

Fig. 1. Northern Blot analysis of mouse tissues. Left panel: 0.5–5 μg of poly $(A)^+$ RNA isolated from adult mouse tissues and the embryonal stem cell line CP1, analyzed by Northern blotting. A full-length cDNA probe for *Evi-1* was used. Right panel: 8–12 μg of poly $(A)^+$ RNA was isolated from adult mouse tissues and was analyzed by Northern blotting, using a 1.2 kb *Sph*I fragment of the *Evi-1* cDNA, which is essentially devoid of zinc-finger sequences. The sizes of the observed bands are indicated to the right of each panel. The bottom two panels show hybridization of the same blots with a β-actin probe.

gestation, we performed *in situ* hybridization analysis of sections of paraformaldehyde-fixed, paraffin-embedded 9.5, 12.5, and 14.5 day mouse embryos. Anti-sense ^{35}S-radiolabeled *Evi-1* probe was prepared from a 454 bp *Bgl*II–*Hin*dIII fragment of the *Evi-1* cDNA (bp 287–741; Morishita *et al.* 1988), which contains 221 bp of 5′ noncoding sequence, and the first 233 bp of coding sequence. To rule out the possibility of cross-hybridization to other sequences, this *Bgl*II–*Hin*dIII fragment was used to probe Southern blots of mouse DNA, and only *Evi-1* fragments were detected (data not shown). To control for nonspecific hybridization of probe to the tissue sections, an unrelated probe (*dilute*) was hybridized to adjacent sections. The pattern of expression seen with this probe showed no overlap with that observed for the *Evi-1* probe (data not shown).

Evi-1 expression was confined to a few distinct organ systems, primarily mesoderm-derived: the urinary system throughout its development; the limb buds during early stages of growth; the endocardial cushion and the conal and truncal ridges of the heart; the bronchioles of the lung; and very localized regions of the nasal cavities. Each of these areas of *Evi-1* expression is considered in detail below.

Expression of Evi-1 *in the developing urinary system*

At 9.5 days *post-coitum* (*p.c.*), the mesonephros is rapidly developing in the lumbar region. Mesonephric tubules extend medially from the mesonephric duct, which develops caudally towards the urogenital sinus. *Evi-1* transcripts were detected throughout the course of the mesonephric duct, as well as in mesonephric tubules (Fig. 2A,B). At 12.5 days *p.c.*, the mesonephros has developed an array of tubules within the urogenital ridge. *Evi-1* expression is high within the mesonephric tubules (Fig. 2C,D), as well as within the Wolffian duct which is the caudal portion of the mesonephric duct (Fig. 3A,B). A distinct ring of expression was seen in the mesenchyme surrounding the more caudal end of the Wolffian duct, a region where the seminal vesicle and prostate will develop in the male.

At 14.5 days *p.c.*, the metanephros is well-formed, and appears as an ovoid structure in the pelvic region, but then shifts cranially over the ensuing days to its final position in the abdominal retroperitoneum. *Evi-1* expression is very high in the tubules of the metanephros at 14.5 days *p.c.* (Fig. 2E,F). No significant expression was detected in the developing adrenal gland (data not shown) or testis (Fig. 2E,F).

Evi-1 is also expressed at significant levels in the adult kidney (Fig. 1). *In situ* hybridization of sections of adult kidney using a nick-translated DNA probe for full-length *Evi-1* revealed a high level of *Evi-1* expression in the tubules throughout the cortex and external medulla (Fig. 2G,H). No expression was detected in the glomeruli.

Evi-1 *expression in the female genital system*

The Mullerian duct develops as an invagination of the coelomic epithelium on the ventrolateral side of the urogenital ridge and can be seen in cross-section in Fig. 3A, ventral to the Wolffian duct. *Evi-1* expression was seen in the Mullerian duct at 12.5 days *p.c.* (Fig. 3B). In females, the Mullerian ducts fuse to form the uterus and the upper third of the vagina. By Northern analysis, we have evidence of *Evi-1* expression in the adult uterus (Fig. 1). *In situ* hybridization of frozen sections of adult uterus with a nick-translated DNA probe for *Evi-1* revealed significant expression in the endometrium (Fig. 3C,D). We also performed immunoperoxidase staining on paraffin sections of formalin-fixed peripubertal and gravid uteri using anti-peptide antiserum against Evi-1, which revealed low but significant amounts of Evi-1 within the nuclei of surface endometrial cells (data not shown).

Evi-1 *expression in the limb bud*

At 9.5 days *p.c.*, when the forelimb bud emerges and is composed of morphologically undifferentiated mesoderm, *Evi-1* expression was clearly seen throughout the non-ectodermal cells of the limb (Fig. 4B). The pattern of *Evi-1* expression in the limb is homogeneous and the apical ectodermal ridge (AER) is negative. *Evi-1* expression was not observed in the somites (Fig. 2A,B; Fig. 4A,B). At 12.5 days *p.c.*, when precartilaginous blastema are forming, homogeneous *Evi-1* expression was observed in the limb (Fig. 4D), similar to that

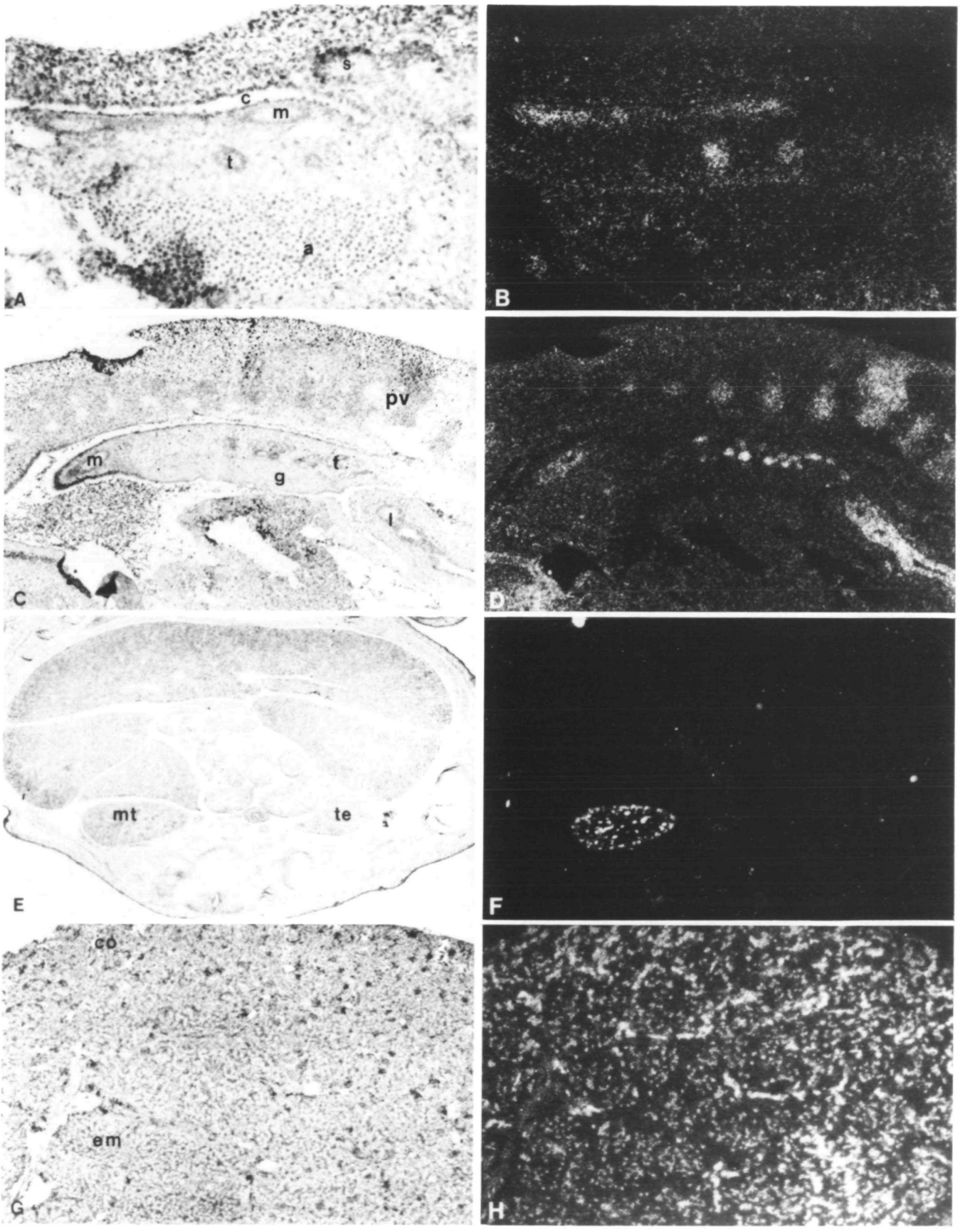
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Fig. 2. *Evi-1* expression in the developing and adult urinary system. *In situ* hybridization of anti-sense ^{35}S-labeled RNA *Evi-1* probe to embryonic (A through F) tissues and *in situ* hybridization of ^{35}S-labeled DNA probe to adult (G and H) tissues. Left-hand panels show bright-field illumination; right-hand panels show the identical field under dark-field illumination. (A and B) Frontal section through a 9.5 days *p.c.* embryo, showing the somites (s), coelomic cavity (c), mesonephric duct (m), and the mesonephric tubules (t). (C and D) Parasagittal section through a 12.5 days *p.c.* embryo, showing the mesonephric duct (m), genital ridge (g), mesonephric tubules (t), the caudal end of the lung (l) and prevertebrae (pv). (E and F) Frontal section through a 14.5 days *p.c.* embryo, showing the liver (unlabelled), metanephros (mt), and testis (te). (G and H) Adult kidney, showing the cortex (co) and external medulla (em). Magnification: A and B, 140×; C and D, 56×; E and F, 17.5×; G and H, 56×.

observed at 9.5 days *p.c.* The expression of *Evi-1* is not confined to the limb bud proper, but extends into the lateral mesoderm in the torso, in areas destined to form the musculoskeleton of the limb girdle. By 14.5 days *p.c.*, the cartilage is well-formed but not yet ossified, and the digits are separated, *Evi-1* expression is limited to the perichondrium of the limb cartilage, both in the forelimb (Fig. 4F) and the footplate (Fig. 4H). In the latter, *Evi-1* expression was seen in the interdigital region.

The sclerotome-derived prevertebral condensations also exhibited a low but significant level of *Evi-1* expression at both 12.5 (Fig. 2D) and 14.5 days *p.c.* (Fig. 5B).

Evi-1 *expression in the heart*

At 12.5 days *p.c.*, the ventricles are partially separated into left and right, and the atria and ventricles are distinct. The truncus arteriosus, the single outflow vessel at this stage, is being divided by rapidly growing opposing ridges of mesenchyme arising on the right superior wall and the left inferior wall of the vessel. These ridges, termed the truncal (more distally located) and conal (more proximally located) ridges, are composed of loose mesenchyme similar in histological appearance to the endocardial cushions, which contrib-

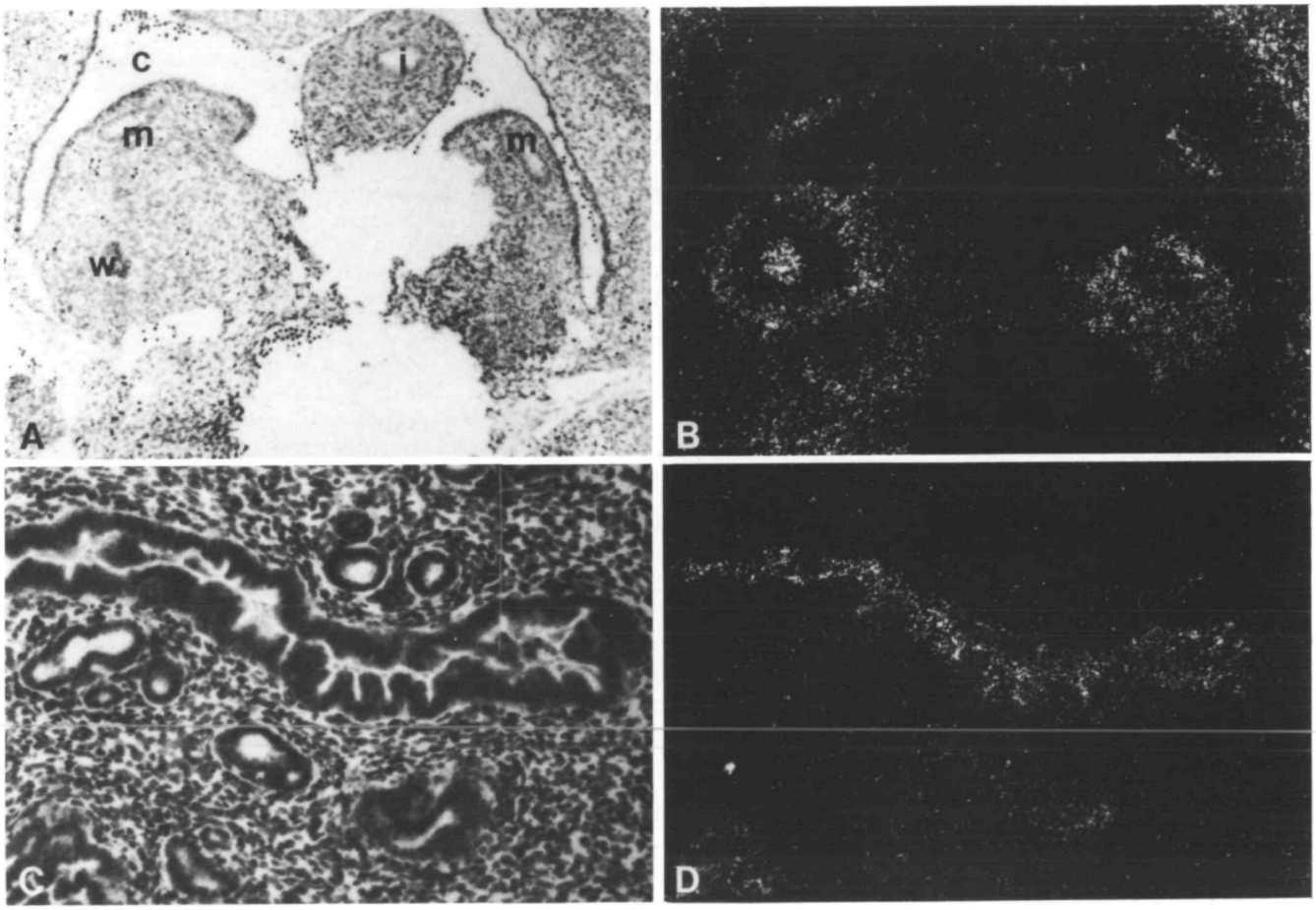

Fig. 3. *Evi-1* expression in the female genital system detected by *in situ* hybridization. (A and B) Bright-field and corresponding dark-field view of a frontal section through a 12.5 days *p.c.* embryo, hybridized with ^{35}S-labeled RNA probe for *Evi-1*, showing the urogenital ridges. Abbreviations: coelomic cavity (c), Mullerian ducts (m), Wolffian duct (w) and intestinal epithelium (i). (C and D) Bright-field and corresponding dark-field view of adult uterus, hybridized with ^{35}S-labeled DNA probe for *Evi-1*, showing the endometrial epithelium and surrounding stroma and glands. Magnification: 150×.

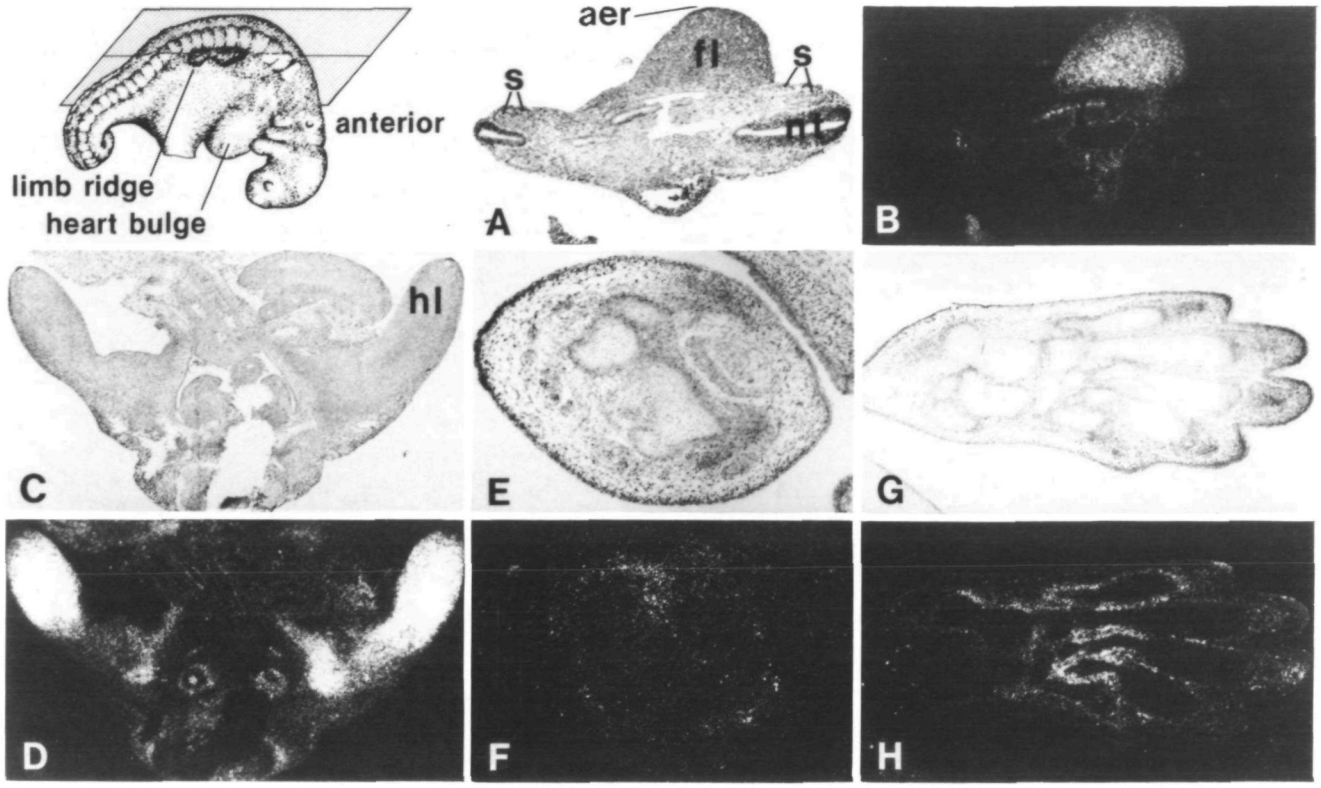

Fig. 4. *Evi-1* expression in the limb buds. *In situ* hybridization of mouse embryos using anti-sense ^{35}S-labeled *Evi-1* probe. (A and B) Upper left panel diagrams a 9.5 days *p.c.* embryo, indicating the plane of section used for panels A and B. These sections show the forelimb bud (fl), somites (s), the neural tube (nt) and the apical ectodermal ridge (aer). (C and D) Frontal section of a 12.5 days *p.c.* embryo, showing the posterior half, including the hind limb (hl). (E and F) Cross section through the forelimb bud of a 14.5 days *p.c.* embryo, with centrally located cartilage anlage of the bones. The lower panel shows a dark-field view of the same field, with hybridization to the perichondrium. (G and H) Section through the footplate of a 14.5 days *p.c.* embryo. Magnification: A and B, 38×; C and D, 24×; E and F, 60×; G and H, 24×.

ute to some of the septa and valves of the heart. Both the truncal and conal ridges, as well as the cardiac valves, exhibited high levels of *Evi-1* expression, whereas the surrounding endocardium, myocardium and blood vessels were apparently negative (Fig. 5B). At 14.5 days *p.c.* (Fig. 5D), no significant *Evi-1* expression was observed in the heart.

Evi-1 *expression in the respiratory system*

We first observed *Evi-1* transcripts in the developing lung at 12.5 days *p.c.*, when the lung is a rapidly developing set of bronchi generated by dichotomous division (Fig. 5B). It appears that both the tracheal and bronchial epithelia contain *Evi-1* transcripts. The surrounding mesenchyme and the visceral pleura appeared negative, as did the adjacent esophagus and the epithelium of the oral cavity, indicating that *Evi-1* expression is highly restricted within these foregut-derived structures. At 14.5 days *p.c.* (Fig. 5D), the lung is the organ with the most intense signal from the *Evi-1* probe. The labeling continues to outline the developing trachea, bronchi and bronchioles. In the adult lung the presence of *Evi-1* transcripts was clearly demonstrated by Northern blot analysis (Fig. 1). Thus, like the kidney, *Evi-1* expression continues through the embryonic and fetal stages into the adult lung. Of note, as shown in Fig. 5B, *Evi-1* expression was also seen at 12.5 days *p.c.* in two derivatives of the visceral arches: the mandible, located below the tongue, and the hyoid cartilage, in the area ventral to the pharynx.

Evi-1 *expression in the developing nasal cavity*

At 12.5 days *p.c.*, while the nasal chambers are deepening, a medial invagination of the nasal epithelium develops bilaterally which will form the vomeromedial organ of Jacobson (Theiler, 1989; Arey, 1966). This organ is innervated by fibers from the first cranial nerve and serves as an additional taste sensor in rodents (Arey, 1966). As shown in Fig. 6, a high level of *Evi-1* expression was detected in the epithelial layer in this invagination, which is undergoing rapid proliferation and invasion into the underlying mesenchyme. In addition, there is a very localized region of mesenchyme lateral to each nasal cavity that exhibited a significant level of staining with *Evi-1* probe. This labeling was found at a fold in the lateral wall of the nasal chamber, and may coincide with the formation of conchae.

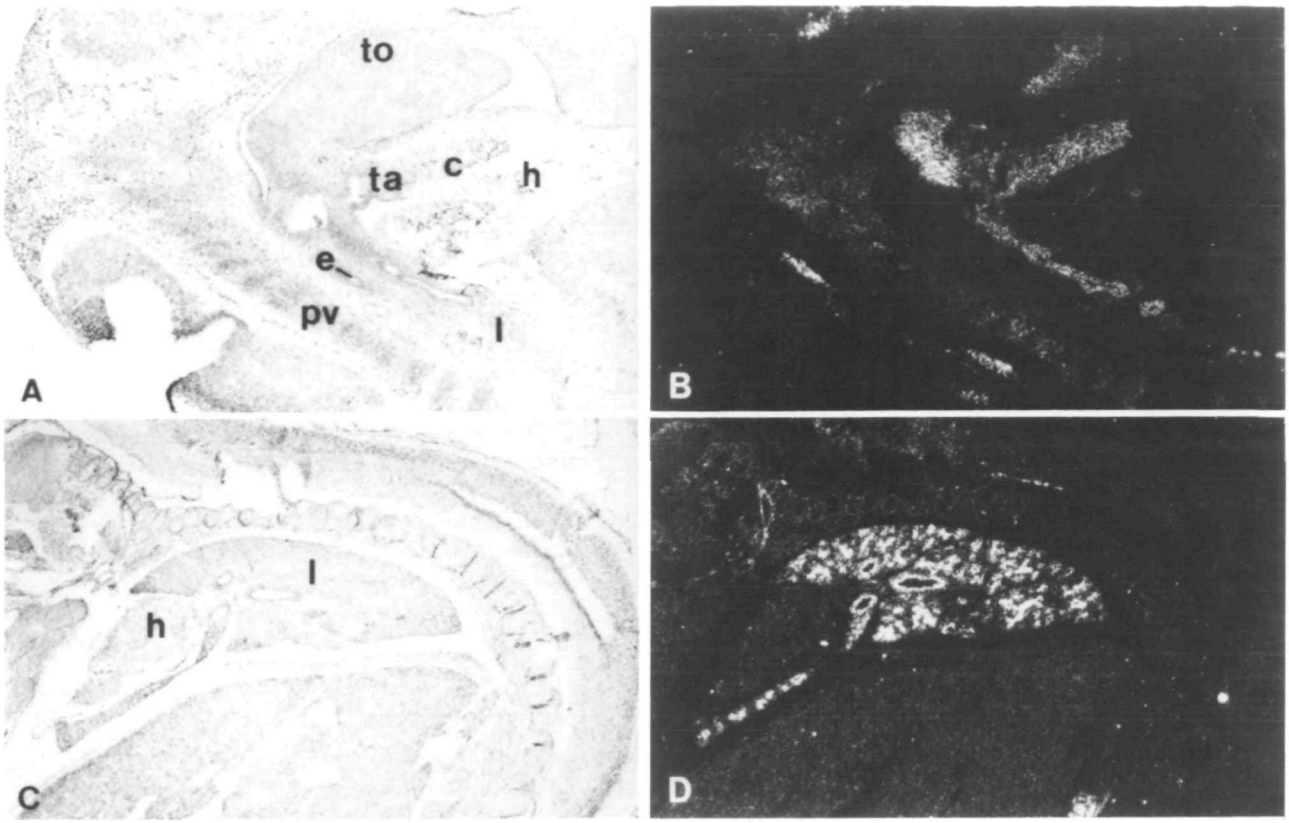

Fig. 5. *Evi-1* expression in the developing lung and heart. (A and B) Parasagittal section through a 12.5 days *p.c.* embryo, showing the tongue (to), truncus arteriosus (ta) and conus (c) of the heart (h), esophagus (e), prevertebrae (pv), and the lung (l). (C and D). Parasagittal section of a 14.5 days *p.c.* embryo, showing the lung (l) and the heart (h). Magnification: A and B, 38×; C and D, 20×.

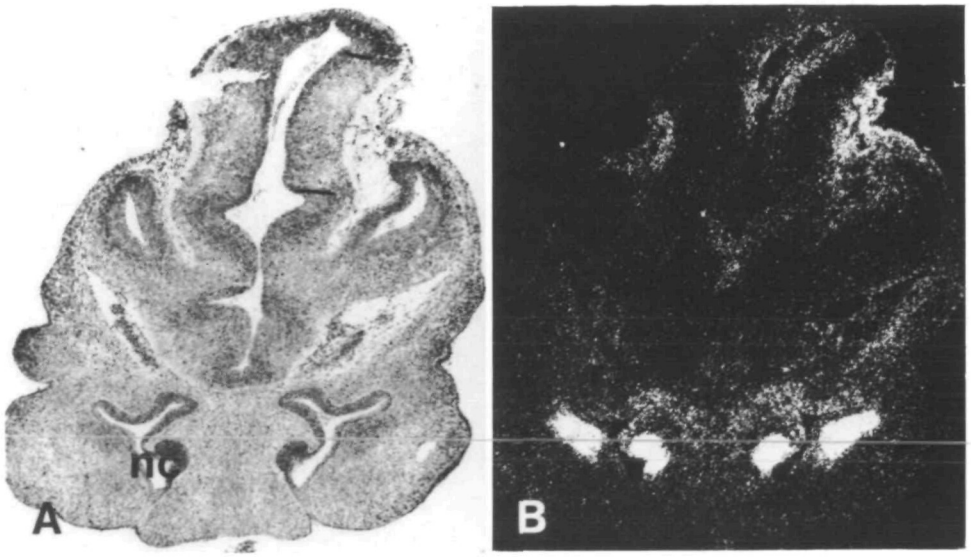

Fig. 6. *Evi-1* expression in the nasal cavities. Frontal section through the head of a 12.5 days *p.c.* embryo, showing the nasal cavities (n.c.). Magnification: 50×.

Discussion

We have examined the extent of *Evi-1* expression in adult and embryonic tissues using Northern blot analysis and *in situ* hybridization. The results indicate that *Evi-1* has a unique spatial and temporal pattern of expression in the developing mouse embryo, and suggest that *Evi-1* plays an important role in key morphogenetic events in several tissues. From our *in situ* hybridization data, it appears that high levels of *Evi-1* expression are restricted in development to a few regions such as kidney, lung, uterus, heart and nasal passages; it is possible, however, that a low level of *Evi-1* expression occurs throughout the embryo since

exposed grains of emulsion are present over almost all organs. Certainly this is true in the adult, where Northern blot analyses revealed *Evi-1* expression in several tissues (Fig. 1; data not shown).

Interestingly, our analysis revealed that the pattern of *Evi-1* expression overlaps significantly with that of other putative or proven transcriptional regulatory proteins. In the limb, *Evi-1* expression overlaps with *Hox-1.1* (Mahon *et al.* 1988), *Hox-5.2* and *Hox-5.3* (Dollé *et al.* 1989), and *Hox-7* (Hill *et al.* 1989; Robert *et al.* 1989) as well as the retinoic acid receptors α, β, and γ (Dollé *et al.* 1989). *Evi-1* is expressed throughout the development of the urinary system, exclusively in the epithelial components, similar to the pattern of expression observed with *Hox-2.1* (Krumlauf *et al.* 1987) and *Hox-5.2* (Dollé and Duboule, 1989). The expression of *Evi-1* transcripts in the endocardial cushions and in the conal and truncal ridges is coincident with the presence of transforming growth factor-beta (TGF-β; Heine *et al.* 1987) and *Hox-7* (Robert *et al.* 1989). These endothelial-derived outgrowths, which are areas of active growth, modeling and remodeling, play a key role in dividing the heart and its outflow tract into separate compartments, and in the formation of the valves. These coincident patterns are consistent with at least two possibilities. One is that *Evi-1* acts in a mutually dependent manner with other genes such as homeobox (*Hox*) or retinoic acid receptor (*RAR*) genes to regulate the expression of subordinate genes. The other possibility is that *Evi-1* plays a direct role in regulating the expression of genes important in development such as *Hox* or *RAR* genes and that these latter genes regulate the expression of subordinate genes. In support of the second possibility, *Krox-20*, a zinc-finger gene with a segment-specific pattern of expression, which overlaps with that of *Hox-1.4*, was found to encode a transcription factor with binding sites in the promoter region of the *Hox-1.4* gene (Chavrier *et al.* 1990). This suggests that zinc-finger genes may function in the mouse to control the transcription of other regulatory genes involved in development. These results (Chavrier *et al.* 1990) also illustrate the validity of using spatial and temporal overlaps in gene expression as clues in determining genetic interrelationships between developmentally important genes. Further experiments, such as finding the target sequences and genes that Evi-1 protein acts upon, and genetic experiments to alter the expression of *Evi-1* in whole animals will be required to resolve the true role of *Evi-1* in development.

In the lung and kidney, an interesting reciprocal relationship is found between the pattern of *Evi-1* expression, which is confined to the epithelial structures, and the pattern of expression of several *Hox* genes which are expressed in the surrounding mesenchyme. Analysis of embryos revealed that *Evi-1* is expressed throughout the development of the urinary system, primarily in the epithelial ducts and tubules. Interestingly, certain *Hox* genes are expressed primarily in the non-epithelial component of the mesonephric and metanephric parenchyma (e.g. *Hox-1.3* (Dony and Gruss, 1987; Gaunt *et al.* 1988), *Hox-1.2, -1.4, -3.1* and *Hox-6.1* (Gaunt *et al.* 1988), and *Hox-1.5* (Gaunt, 1988)). An analogous localization of *Evi-1* expression to epithelial structures and *Hox* gene expression to adjacent mesenchyme is present in the developing lung. It is clear from these studies that *Evi-1* is expressed at significant levels in the foregut-derived epithelium of the trachea, bronchi and bronchioles (Fig. 6). The visceral pleura, and the mesenchyme surrounding the developing bronchioles, which are derived from the splanchnic mesoderm, appear negative. This pattern of expression is the reciprocal of the pattern observed for *Hox-1.2, 1.4, 1.5,* and *Hox-2.1* (Gaunt *et al.* 1988; Holland and Hogan, 1988; Krumlauf *et al.* 1987). In both the kidney and the lung, an inductive interrelationship exists between the mesenchyme and epithelium (Saxén *et al.* 1986; Taderera, 1967). As suggested by Holland and Hogan (1988), it is possible that these regulatory genes may play an important role in these interactions.

Our studies on *Evi-1* suggest several specific developmental processes in which *Evi-1* may play an important role, such as renal and pulmonary organogenesis and limb development. These possibilities can be probed genetically in transgenic mice by attempting to create dominant-acting gain-of-function mutations by directing high levels of *Evi-1* expression to inappropriate tissues or mutant forms of *Evi-1* to tissues such as the kidney, lung, or limb, where *Evi-1* is normally expressed. Recent experiments of this type have helped to delineate the role of *Hox-1.1* in development (Kessel *et al.* 1990). Also, loss-of-function mutations generated by homologous recombination may provide important clues into the function of *Evi-1* in mouse development.

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References

Arey, L. B. (1966). *Developmental Anatomy.* W. B. Saunders, Philadelphia, PA.

Aviv, M. and Leder, P. (1972). Purification of biologically active globin messenger RNA by chromotography on oligothymidilic acid-cellulose. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1408–1412.

Chavrier, P., Vesque, C., Galliot, B., Vigneron, M., Dollé, F. Duboule, D. and Charnay, P. (1990). The segment-specific gene *Krox-20* encodes a transcription factor with binding sites in the promoter region of the *Hox* 1.4 gene. *EMBO J.* **9**, 1209–1218.

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

Church, G. M. and Gilbert, W. (1984). Genomic sequencing. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1991–1995.

Dollé, P. and Duboule, D. (1989). Two gene members of the murine *Hox-5* complex show regional and cell-type specific expression in developing limbs and gonads. *EMBO J.* **8**, 1507–1515.

Dollé, P , Ruberte, E., Kastner, P., Petkovich, M., Stoner, C.

M., GUDAS, L. J. AND CHAMBON, P. (1989). Differential expression of genes encoding of β and γ retinoic acid receptors and CRABP in the developing limbs of the mouse. *Nature* **342**, 702–705.

DONY, C. AND GRUSS, P. (1987). Specific expression of the *Hox* 1.3 homeo box gene in murine embryonic structures originating from or induced by the mesoderm. *EMBO J.* **6**, 2965–2975.

GAUNT, S. J. (1988). Mouse homeobox gene transcripts occupy different but overlapping domains in embryonic germ layers and organs: a comparison of *Hox-3.1* and *Hox-1.5*. *Development* **103**, 135–144.

GAUNT, S. J., SHARPE, P. T. AND DUBOULE, D. (1988). Spatially restricted domains of homeogene transcripts in mouse embryos: relation to a segmented body plan. *Development* **104 Supplement**, 169–179.

HAASE, A. T., STOWRING, L., HARRIS, J. D., TRAYNOR, B., VENTURA, P., PELUSO, R. AND BRAHIC, M. (1982). Visna DNA synthesis and the tempo of infection *in vitro*. *Virology* **119**, 399–410.

HEINE, U., MUNOZ, E. F., FLANDERS, K. C., ELLINGSWORTH, L. R., LAM, H.-Y. P., THOMPSON, N. L., ROBERTS, A. B. AND SPORN, M. B. (1987). Role of transforming growth factor-β in the development of the mouse embryo. *J. Cell Biol.* **105**, 2861–2876.

HILL, R. E., JONES, P. F., REES, A. R., SIME, C. M., JUSTICE, M. J., COPELAND, N. G., JENKINS, N. A., GRAHAM, E. AND DAVIDSON, D. R. (1989). A new family of mouse homeo box-containing genes: molecular structure, chromosomal location, and developmental expression of *Hox-7.1*. *Genes and Dev.* **3**, 26–37.

HOLLAND, P. W. H. AND HOGAN, B. L. M. (1988). Spatially restricted patterns of expression of the homeobox-containing gene *Hox-2.1* during mouse embryogenesis. *Development* **102**, 159–174.

KESSEL, M., BALLING, R. AND GRUSS, P. (1990). Variations of cervical vertebrae after expression of a *Hox-1.1* transgene in mice. *Cell* **61**, 301–308.

KRUMLAUF, R., HOLLAND, P. W., MCVEY, J. H. AND HOGAN, B. L. M. (1987). Developmental and spatial patterns of expression of the mouse homeobox gene, *Hox 2.1*. *Development* **99**, 603–617.

MAHON, K. A., WESTPHAL, H. AND GRUSS, P. (1988). Expression of homeobox gene *Hox 1.1* during mouse embryogenesis. *Development* **104 Supplement**, 187–195.

MATSUGI, T., MORISHITA, K. AND IHLE, J. N. (1990). Identification, nuclear localization and DNA-binding activity of a zinc-finger protein encoded by the *Evi-1* myeloid transforming gene. *Molec. cell. Biol.* **10**, 1259–1264.

MCMASTER, G. K. AND CARMICHAEL, G. G. (1977). Analysis of single and double-stranded nucleic acids on polyacrylamide gels and agarose gels by using glyoxal and acridine orange. *Proc natn. Acad. Sci. U.S.A.* **74**, 4835–4838.

MILLER, J., MCLACHLAN, A. D. AND KLUG, A. (1985). Repetitive zinc-binding domains in the protein transcription factor III A from *xenopus* oocytes. *EMBO J.* **4**, 1609–1614.

MORISHITA, K., PARGANAS, E., DOUGLASS, E. C. AND IHLE, J. N. (1990). Unique expression of the human *Evi-1* gene in an endometrial carcinoma cell line: sequence of cDNAs and structure of alternatively spliced transcripts. *Oncogene* **5**, 963–972.

MORISHITA, K., PARKER, D. S., MUCENSKI, M. L., JENKINS, N. A., COPELAND, N. G. AND IHLE, J. N. (1988). Retroviral activation of a novel gene encoding a zinc finger protein in IL-3-dependent myeloid leukemia cell lines. *Cell* **54**, 831–840.

MUCENSKI, M. L., TAYLOR, B. A., IHLE, J. N., HARTLEY, J. W., MORSE, H. C. III, JENKINS, N. A. AND COPELAND, N. G. (1988). Identification of a common ecotropic viral integration site, *Evi-1*, in the DNA of AKXD murine myeloid tumors. *Molec. cell. Biol.* **8**, 301–308.

OLIVER, G., SIDELL, N., FISKE, W., HEINZMANN, C., MOHANDAS, T., SPARKES, R. S. AND DEROBERTIS, E. M. (1989). Complementary homeoprotein gradients in developing limb buds. *Genes and Dev.* **3**, 641–650.

PERKINS, A. S., KIRSCHMEIER, P., GATTONI-CELLI, S. AND WEINSTEIN, I. B. (1983). Design of a retrovirus derived vector for the expression and transduction of exogenous genes in mammalian cells. *Molec. cell. Biol.* **3**, 1123–1132.

RIGBY, P. W. J., DIECKMANN, M., RHODES, C. AND BERG, P. (1977). Labeling of DNA to high specific activity by nick translation. *J. molec. Biol.* **113**, 237–258.

ROBERT, B., SASSOON, D., JACQ, B., GEHRING, W. AND BUCKINGHAM, M. (1989). *Hox-7*, a mouse homeobox gene with a novel pattern of expression during embryogenesis. *EMBO J.* **8**, 91–100.

RUBERTE, E., DOLLÉ, P., KRUST, A., ZELENT, A., MORRISS-KAY, G. AND CHAMBON, P. (1990). Specific spatial and temporal distribution of retinoic acid receptor gamma transcripts during mouse embryogenesis. *Development* **108**, 213–222.

SASSOON, D. A., GARDNER, I. AND BUCKINGHAM, M. (1988). Transcripts of α-cardiac and α-skeletal actins are early markers for myogenesis in the mouse embryo. *Development* **104**, 155–164.

SAXÉN, L., SARIOLA, H. AND LEHTONEN, E. (1986). Sequential cell and tissue interactions governing organogenesis of the kidney. *Anat. Embryol.* **175**, 1–6.

STRUHL, K. (1987). Promoters, activator proteins and the mechanism of transcriptional initiation in yeast. *Cell* **49**, 295–297.

TADERERA, J. V. (1967). Control of lung differentiation *in vitro*. *Devl Biol.* **16**, 489–512.

THEILER, K. (1989). *The House Mouse*. In *Atlas of Embryonic Development*. Springer-Verlag, New York.

THOMAS, P. S. (1980). Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. natn. Acad Sci. U.S.A.* **77**, 5201–5205.

WAHL, G. M., STERN, M. AND STARK, G. R. (1979). Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxy methyl paper and rapid hybridization using dextran sulphate. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3683–3687.

WILKINSON, D. G., BAILES, J. A., CHAMPION, J. E. AND MCMAHON, A. P. (1987). A molecular analysis of mouse development from 8 to 10 days *post coitum* detects changes only in embryonic globin expression. *Development* **99**, 493–500.

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