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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*

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Summary

By use of *in situ* hybridization experiments, the mouse homeobox genes *Hox-3.1* and *Hox-1.5* are compared in the temporal and spatial patterns of their embryonic transcripts. Transcripts of both genes are first detected at about 7½ days, although the appearance of *Hox-3.1* transcripts apparently follows *Hox-1.5* after a small delay. *Hox-3.1* and *Hox-1.5* transcripts occupy domains which are different, although overlapping, along the anteroposterior axis of the embryo. The domains are first established within the ectoderm and mesoderm germ layers at 7½–8 days, but subsequently they persist within the nervous system, the prevertebral column and within at least some of the organs (the thyroid, lung, stomach, mesonephric and metanephric kidneys) at 12½ days. In discussion, two different mechanisms are considered by which positional information might first be generated within the germ layers.

Key words: mouse embryo, homeobox genes, transcript domains, germ layers, organs.

Introduction

We found earlier (Gaunt *et al.* 1986) that transcripts of the mouse homeobox gene *Hox-1.5* (Mo-10) were restricted to posterior parts of the ectoderm and mesoderm germ layers in 7½-day embryos. Restriction to posterior ectodermal structures (posterior nervous tissue) and posterior mesodermal structures (such as mesonephric tubules and somites) persisted during organogenesis at 9½ days. In view of these results, and also of the part known to be played by homeobox genes in pattern formation during *Drosophila* embryogenesis (e.g. Gehring, 1987), we suggested that expression of the *Hox-1.5* gene might provide a positional cue during the development of ectoderm- and mesoderm-derived structures in the mouse. This suggestion was supported by the fact that the transcript domain for *Hox-1.5* differed spatially, at least within the nervous system, from that already described for *Hox-3.1* (Mo-EA, Awgulewitsch *et al.* 1986).

From the above and more recent studies, it is becoming apparent that mouse homeobox gene transcripts occupy different, although overlapping, spatial domains in both the nervous system and prevertebrae of midgestation embryos. In these tissues, the homeobox genes characterized have transcript domains which overlap posteriorly, but which extend to different anterior limits. Within the central nervous system, these anterior limits are in the anterior (preotic) myelencephalon (*Hox-1.5*: Gaunt *et al.* 1986; Gaunt, 1987; Fainsod *et al.* 1987), the posterior myelencephalon (for *Hox-2.1*: Krumlauf *et al.* 1987; Utset *et al.* 1987; Holland & Hogan, 1988; for *Hox-1.4* and *Hox-1.2*: Toth *et al.* 1987), the anterior spinal cord (*Hox-6.1*: Sharpe *et al.* 1988) and the cervical spinal cord at the level of the third to fifth prevertebrae (*Hox-3.1*: Awgulewitsch *et al.* 1986; Utset *et al.* 1987; Holland & Hogan, 1988). In the prevertebral column, the anterior limits are inclusive of the first prevertebrae (*Hox-1.5*: Gaunt, 1987), at the level of the seventh to eighth prevertebrae (for *Hox-6.1*: Sharpe *et al.* 1988; for *Hox-1.3*: Dony & Gruss, 1987), and the twelfth prevertebra (*Hox-3.1*: Holland & Hogan, 1988).

In addition to nervous and prevertebral tissue (and the somites of early embryos that give rise to the

prevertebrae), other organs display homeobox gene transcripts in a way that is spatially restricted. This has been demonstrated most clearly for *Hox-1.3* (Dony & Gruss, 1987) and *Hox-2.1* (Holland & Hogan, 1988). It has not yet been established, however, that homeobox gene transcripts within the organs occupy different but overlapping domains. Thus, it remains to be examined whether or not different organs exhibit different arrays of homeobox gene transcripts depending upon their position along the anteroposterior axis of the body.

Although *Hox-1.5* transcripts are first detected in posterior regions of the germ layers at 7½ days (Gaunt, 1987), it is not yet known whether different homeobox genes already occupy different transcript domains at this early time or, indeed, whether different genes commence expression synchronously or sequentially (Gaunt, 1987). In the present paper, comparison is made between *Hox-3.1* and *Hox-1.5* in the temporal and spatial patterns of their embryonic transcripts. These genes were chosen for comparison specifically because their transcript domains provide the most widely separated anterior boundaries. It is now shown that the transcripts of these two genes occupy different, but overlapping, spatial domains both within the germ layers at 7½–8¼ days, and in various organs at 12½ days.

Materials and methods

Preparation of embryo sections

Embryos were obtained from natural matings between F_1 (CBA×C57BL/6) mice. For aging of embryos, midday on the day of the vaginal plug was designated day ½ of pregnancy. Fixation, embedding and sectioning of embryos was as previously described (Gaunt *et al.* 1986; Gaunt, 1987), but sections were cut at a thickness of 7 μm.

Preparation and use of ^{35}S-labelled RNA probes

For *Hox-1.5*, production and alkaline hydrolysis of ^{35}S-labelled RNA probes was as previously described (Gaunt, 1987). The mouse homeobox gene *Hox-3.1* has been cloned by Awgulewitsch *et al.* (1986) and by Breier *et al.* (1986). In addition, *Hox-3.1* genomic DNA has been independently cloned, and the homeobox sequenced, by D. Duboule *et al.* (unpublished data). A clone of *Hox-3.1* genomic DNA in pBR322 was received as a gift from Denis Duboule. From this, the 330 bp *Pst*I–*Ava*I homeobox-containing fragment (Breier *et al.* 1986) was subcloned into pGEM-3 (Promega). This fragment has previously been found to hybridize specifically to *Hox-3.1* mRNA (2·7 kb) in Northern blots (Breier *et al.* 1986). For production of ^{35}S-labelled RNA probes to *Hox-3.1* mRNA (antisense probes), the recombinant plasmid was cut with *Hin*dIII and then used in a T7 RNA polymerase reaction, as already described for *Hox-1.5* (Gaunt *et al.* 1986; Gaunt, 1987). For production of ^{35}S-labelled *Hox-3.1* sense (control) RNA probe, plasmid was cut with *Eco*RI and then used in an SP6 RNA polymerase reaction. Prior to use in hybridizations, *Hox-3.1* RNA probes were hydrolysed at pH 10·2 for 60 min (Cox *et al.* 1984).

Methods used for *in situ* hybridization, RNase A digestion, washing, autoradiography and staining of sections were all as previously described (Gaunt *et al.* 1986).

Results

7½-day embryos

Sagittal and near-sagittal sections were prepared and examined by *in situ* hybridization for *Hox-3.1* and *Hox-1.5* transcripts. During sectioning of an embryo, the midline was indicated by the presence of the allantois at the posterior end. For each embryo at 7½ days only about eight adjacent sections included the allantois, and it was usually found that such a small number of sections readily permitted comparative hybridization of only two different probes. For most embryos, the probes compared were *Hox-3.1* antisense *versus Hox-1.5* antisense. For remaining embryos, and with the inclusion of at least one such embryo per experiment, the probes compared were antisense (*Hox-3.1* or *Hox-1.5*) *versus* sense (*Hox-3.1* or *Hox-1.5*). Sense (control) probes consistently produced only background levels of hybridization, whilst antisense probes on adjacent sections gave specific hybridization, as described below. A comparison of *Hox-1.5* sense *versus* antisense probes hybridized to parallel sections of an 8-day embryo has already been published (Gaunt, 1987).

Figs 1, 2 and 3 illustrate variation in the developmental stage reached by 7½ days. In 'early 7½-day' embryos (Fig. 1), anterior migration of mesoderm had not yet separated completely the ectoderm and endoderm layers, and posterior migration of mesoderm had produced only a small allantois. In 'late 7½-day' embryos (Fig. 2), the first signs of head-fold formation were apparent and the allantois was well developed.

When tested by *in situ* hybridization, early 7½-day embryos (six such embryos were studied) showed no detectable *Hox-3.1* (Fig. 1A) or *Hox-1.5* (Fig. 1B) transcripts. Late 7½-day embryos (five such embryos were studied) showed *Hox-3.1* transcripts localized mainly or entirely within the allantois (Fig. 2A), while *Hox-1.5* transcripts were located both in the allantois and in the ectoderm and mesoderm layers of the posterior half of the embryo (Fig. 2B). Parasagittal sections at this stage (not shown), not including the allantois, showed no *Hox-3.1* transcripts but continued to show *Hox-1.5* transcripts over at least six sections to either side of the allantois. Two of the 7½-day embryos studied, which were at a stage of development intermediate between the 'early' and

'late' embryos, showed *Hox-1.5* transcripts (Fig. 3B) but little or no evidence of *Hox-3.1* transcripts (Fig. 3A). It is therefore probable that *Hox-1.5* transcripts begin to accumulate earlier than *Hox-3.1* transcripts. However, the precise time interval between first expression of these two genes is at present uncertain. It is important to note that in the early stages of *Hox-1.5* expression (Fig. 3B) transcripts were detected over the complete posterior half of the ectoderm and mesoderm layers: no embryos were found in which *Hox-1.5* transcripts, like the earliest-detected *Hox-3.1* transcripts (Fig. 2A), were restricted entirely to the allantois.

8- and 8¼-day embryos

Fig. 4 compares the transcript domains of *Hox-3.1* and *Hox-1.5* at 8 days. In the embryo shown, the head fold was well developed and three somites had already been formed. Transcripts of both genes were seen in posterior regions of ectoderm and mesoderm, including the allantois, but the transcript domain for *Hox-1.5* (Fig. 4B) extended to a more anterior position than that for *Hox-3.1* (Fig. 4A). For *Hox-3.1*, the anterior limits of transcripts within both ectoderm and mesoderm were situated posterior to the position of the earliest-developing somites. For *Hox-1.5*, both within ectoderm and mesoderm tissues, the anterior limits of transcripts extended anterior to the position of first somite formation. It therefore appears likely that the *Hox-1.5* transcript domain within mesoderm tissue includes somite one.

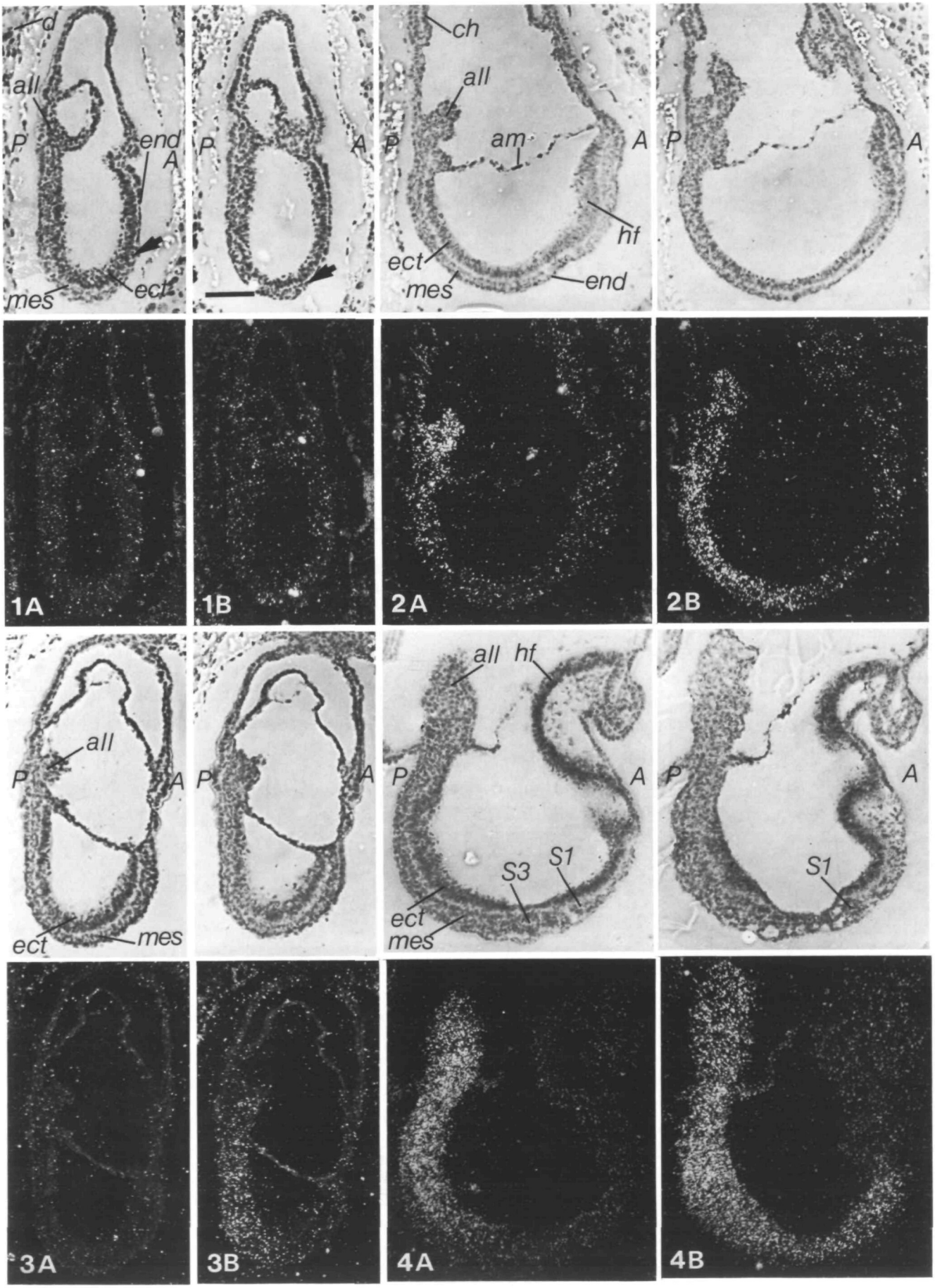
Fig. 5 compares the transcript domains of *Hox-3.1* and *Hox-1.5* in an 8-somite-stage (8¼-day) embryo. At this stage, it was apparent that the *Hox-1.5* transcript domain (Fig. 5B) extended anteriorly into that part of the neural ectoderm which was becoming segmented as neuromeres, and which would normally have formed the hindbrain (Rugh, 1968). Although not shown very precisely in Fig. 5B, the anterior limit of transcripts was usually found at this stage to coincide with a neuromere constriction on the ventral surface of the neural ectoderm (Gaunt *et al.* 1986; Gaunt, 1987). The *Hox-1.5* transcript domain within mesoderm included somites two to eight (Fig. 5B) although, as previously described (Gaunt, 1987), the intensity of labelling within mesoderm was now becoming less than that in the neural ectoderm. In a parallel section of the same embryo, *Hox-3.1* transcripts were clearly restricted to ectoderm and mesoderm posterior to the region of early somite formation (Fig. 5A).

The 12½-day embryo

Fig. 6 shows a comparison of *Hox-3.1* and *Hox-1.5* transcript domains as detected by *in situ* hybridization on adjacent or nearby sections taken from the same 12½-day embryo. The anterior boundaries of these domains within the nervous system at about this stage have already been described elsewhere (for *Hox-3.1*: Awgulewitsch *et al.* 1986; Utset *et al.* 1987; Holland & Hogan, 1988; for *Hox-1.5*: Gaunt, 1987; Fainsod *et al.* 1987), and are indicated here by large arrows on Fig. 6A. For *Hox-3.1*, the boundary is at the level of the fifth prevertebra in the ventral third of the spinal cord, and at about the level of the third prevertebra in the centre of the spinal cord. For *Hox-1.5*, the boundary is within the preotic hindbrain. For both genes, there is a gradual anterior-to-posterior reduction in the intensity of labelling along the length of the spinal cord (not shown).

Fig. 6C and D compare *Hox-3.1* and *Hox-1.5* transcripts in tissues that surround the pharynx. No specific labelling of this region was given by the *Hox-3.1* probe (Fig. 6C), but *Hox-1.5* transcripts were clearly present in the most anterior prevertebrae, including prevertebra 1 (Fig. 6D). The thyroid duct (Fig. 6A) leads from the pharynx to the thyroid gland which, at 12½ days, lies at the base of the duct, deep in the pharyngeal floor (Fig. 6A; Rugh, 1968). Strong labelling with the *Hox-1.5* probe was detected over the thyroid gland (Fig. 6D). Less-intense labelling with this probe was also seen generally over tissue which formed the floor of the pharynx (Fig. 6D). Labelling of the pharyngeal floor extended anterior to the thyroid duct, but did not extend over the tongue. The thyroid gland consists of both endoderm- and mesoderm-derived components (Hilfer, 1968) but sections of thyroid tissue at 12½ days did not permit clear distinction between these cell types. In some sections, although not apparent in Fig. 6D, the duct leading into the thyroid from the pharynx showed only low levels of labelling when compared with the surrounding tissue. Posterior to the thyroid region, labelling with the *Hox-1.5* probe extended to include a small area of tissue in the most anterior (atrial) region of the heart (not shown). Most of the heart tissue, however, showed only background labelling after hybridization with the *Hox-1.5* probe. No specific labelling of any part of the heart was given by the *Hox-3.1* probe (not shown).

Fig. 6E and F compare *Hox-3.1* and *Hox-1.5* transcripts in prevertebrae 10–14 and adjacent tissues. *Hox-3.1* transcripts were seen in prevertebra 12 but not in any of the more anterior prevertebrae (Fig. 6E). Prevertebrae 13–16 were labelled strongly by the *Hox-3.1* probe, and labelling persisted at a lower intensity over all more posterior prevertebrae. This finding is similar to that described for *Hox-3.1* by Holland & Hogan (1988). All prevertebrae were labelled by the *Hox-1.5* probe, and Fig. 6F shows *Hox-1.5* transcripts in prevertebrae 10–14. The lung was positive for *Hox-1.5* (Fig. 6F) but not *Hox-3.1*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transcripts (Fig. 6E). *Hox-1.5* transcripts in the lung were restricted to mesodermal components and were not detected in the endodermally-derived lining epithelium. This distribution is therefore similar to that already described for transcripts of *Hox-2.1* (Krumlauf *et al.* 1987), *Hox-1.3* (Dony & Gruss, 1987) and *Hox-6.1* (Sharpe *et al.* 1988).

Fig. 6G,H compare *Hox-3.1* and *Hox-1.5* transcripts in the liver, stomach, mesonephric and metanephric kidneys. Neither gene was seen to be ex-

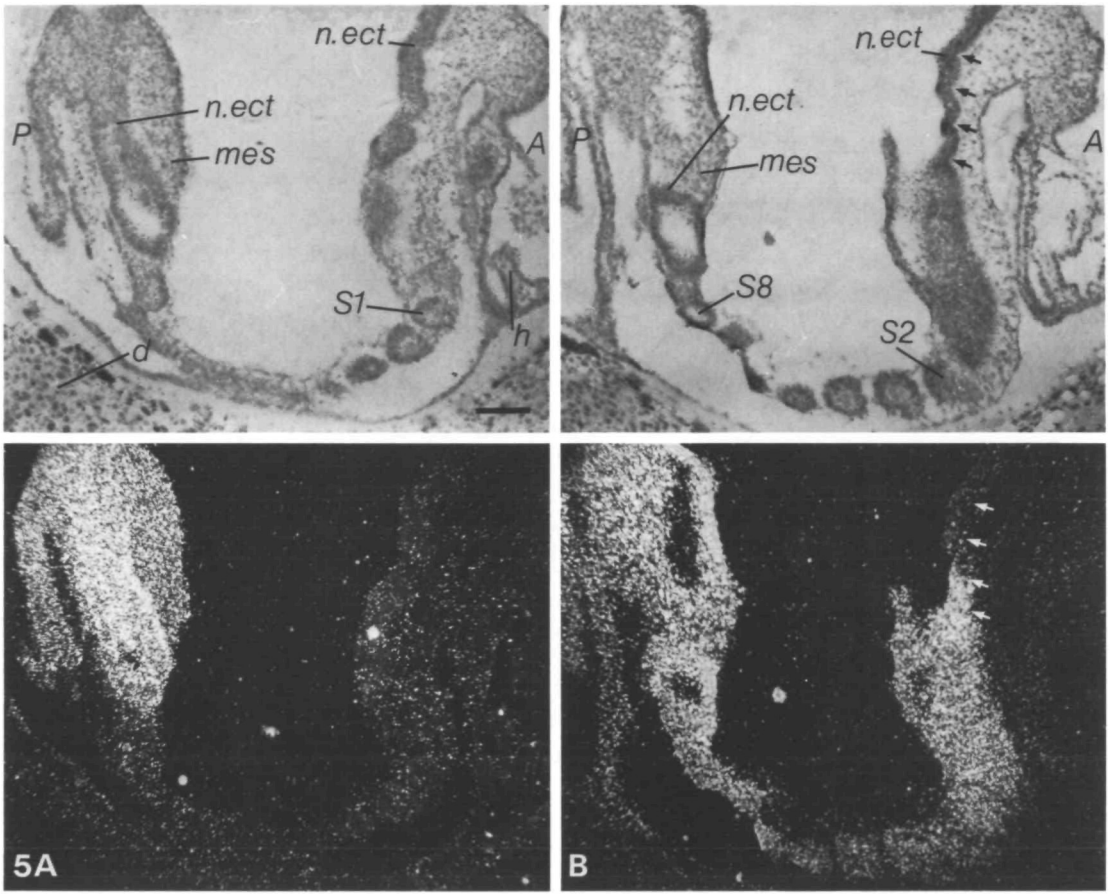
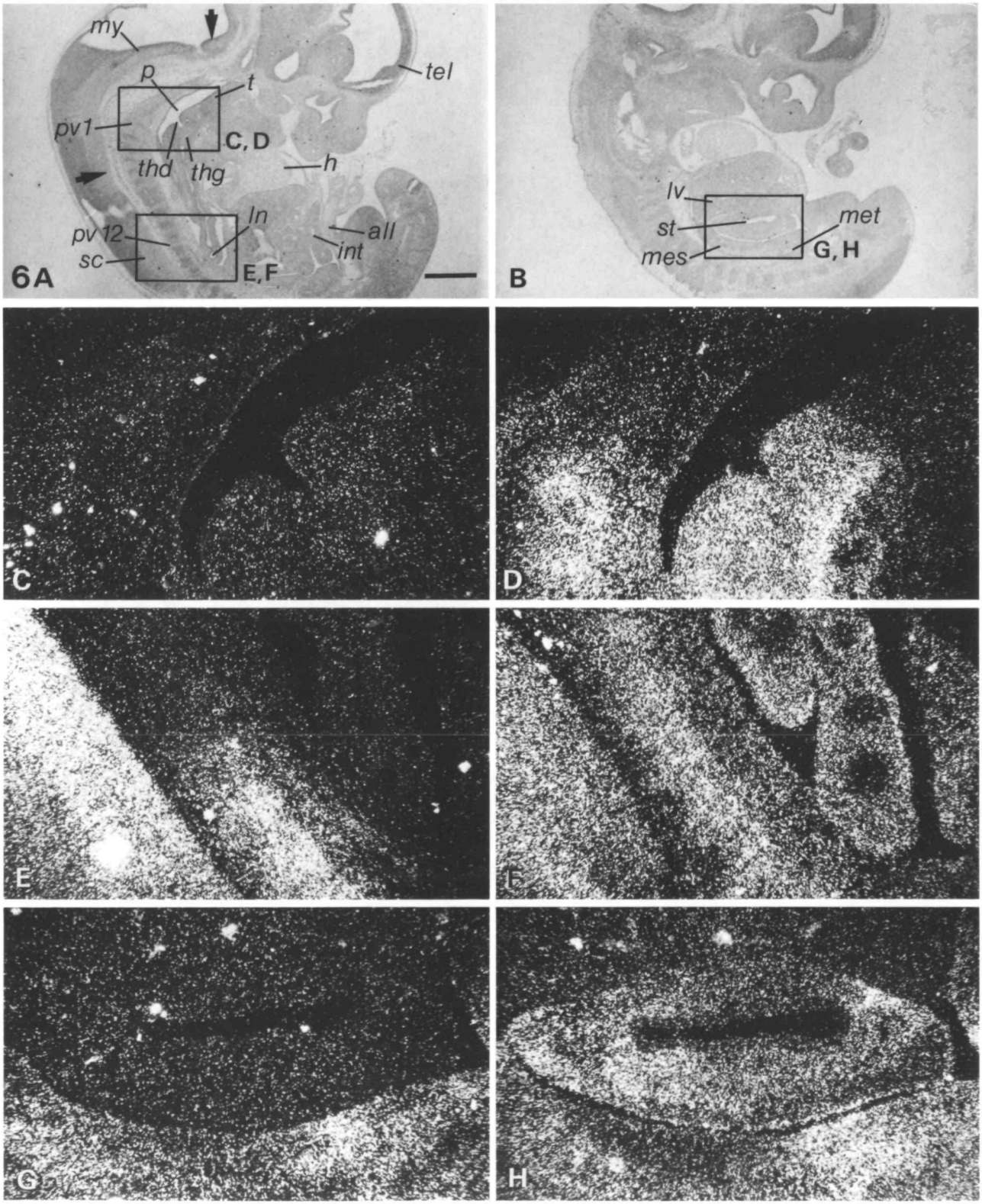


Fig. 5. Expression of *Hox-3.1* (Fig. 5A) and *Hox-1.5* (Fig. 5B) as detected in nearby sections of an 8-somite (8¼-day) embryo. Sections are near-sagittal. Upper panels, phase contrast; lower panels, dark-field illumination. *n. ect*, neural ectoderm; *S1*, *S2*, *S8*, somites 1, 2 and 8; *h*, heart; arrows in Fig. 5B, constrictions between neuromeres; other labels as for Figs 1–4. Bar, 0·1 mm. The *Hox-3.1* transcription domain was restricted to neural ectoderm and mesoderm tissues posterior to the level of somite eight (Fig. 5A). The *Hox-1.5* transcription domain extended anteriorly into the hindbrain (characterized by neuromeres) and included somites two to eight (Fig. 5B).

Figs 1–4. Expression of *Hox-3.1* and *Hox-1.5* in 7½-day (Figs 1, 2, 3) and 8-day (Fig. 4) embryos. Sections are sagittal or near-sagittal. Upper panels, phase contrast; lower panels, dark-field illumination. For each embryo, adjacent or nearby sections were hybridized to either *Hox-3.1* (Figs 1A–4A) or *Hox-1.5* (Figs 1B–4B) probes. *A*, anterior; *P*, posterior; *d*, decidual tissue; *ect*, ectoderm; *mes*, mesoderm; *end*, endoderm; arrows in Fig. 1 indicate the anterior limit of the advancing mesoderm layer; *all*, allantois; *am*, amnion; *ch*, chorion; *hf*, head fold; *S1*, *S3*, somites 1 and 3. Bar (applies to all embryos), 0·1 mm. No expression was detected in 'early' 7½-day embryos (Fig. 1A,B). In 'late' 7½-day embryos, *Hox-3.1* transcripts were located mainly within the allantois (Fig. 2A) whilst *Hox-1.5* transcripts were found in the ectoderm and mesoderm of the posterior half of the embryo (Fig. 2B). *Hox-1.5* transcripts first became detectable before *Hox-3.1* transcripts (cf. Fig. 3B with A). At 8 days, the anterior limits of the transcription domains were posterior (for *Hox-3.1*; Fig. 4A) and anterior (for *Hox-1.5*; Fig. 4B) to the region of somite formation.

pressed in the liver, but both *Hox-3.1* and *Hox-1.5* transcripts were detected in the mesonephric and metanephric kidneys. These findings for the kidney are as earlier reported by Awgulewitsch *et al.* (1986) for *Hox-3.1*, and by Gaunt *et al.* (1986) for *Hox-1.5*. The distributions of *Hox-3.1* and *Hox-1.5* transcripts in the kidney were similar, with no obvious spatial restriction within the tissue. The stomach was positive for *Hox-1.5* (Fig. 6H) but not *Hox-3.1* transcripts (Fig. 6G). As noted above for the lung, *Hox-1.5*



transcripts in the stomach were restricted to mesodermal components and were not detected in the endodermally-derived lining epithelium. A similar pattern of distribution within the stomach has been reported for transcripts of *Hox-2.1* (Holland & Hogan, 1988) and *Hox-1.3* (Dony & Gruss, 1987).

Discussion

In this paper, the mouse homeobox genes *Hox-3.1* and *Hox-1.5* are compared in the temporal and spatial patterns of their embryonic transcripts. Transcripts of both genes were first detected by *in situ* hybridization at about 7½ days, although the appearance of *Hox-3.1* transcripts apparently followed *Hox-1.5* after a small delay. *Hox-3.1* and *Hox-1.5* transcripts occupied different, but overlapping, domains along the anteroposterior axis of the embryo. This was found both within the ectoderm and mesoderm germ layers at 7½–8¼ days, and in at least some of the organs at 12½ days. These results extend earlier findings that *Hox-3.1* and *Hox-1.5* transcripts occupy different but overlapping domains within the nervous system and prevertebrae of midgestation embryos (for *Hox-3.1*: Utset *et al.* 1987; Holland & Hogan, 1988; for *Hox-1.5*: Gaunt *et al.* 1986; Gaunt, 1987; Fainsod *et al.* 1987). At the present time, the term 'overlapping' used here to describe transcript domains is only strictly applicable to gross regions of tissue such as, for example, an area of the spinal cord, prevertebral column or kidney. It seems likely that transcript domains of mouse homeobox genes also overlap within individual cells, but it is important to note that this is not yet proven.

Table 1. *Homeobox gene expression in organs of midgestation embryos*

	Homeobox gene (anteriormost prevertebra showing expression)		
	Hox-1.5 (1st)	*Hox-6.1** (7th)	*Hox-3.1* (12th)
Heart	–	–	–
Thyroid	+	–	–
Lung	+	+	–
Stomach	+	+	–
Kidney	+	+	+

The heart, lung, stomach and kidney are listed in a sequence which probably corresponds to the relative position along the anteroposterior axis of their founder cells in the mesoderm germ layer at the time of cellular determination (for references, see Holland & Hogan, 1988). The thyroid gland develops around an evagination of the foregut (in the pharyngeal region at the level of the first to second visceral arches) which is situated posterior to the origins of the heart (Rugh, 1968), but anterior to that part of the foregut which first contributes to the developing lung bud (Patten, 1958). The mesodermal component of the thyroid, unlike that of the other organs listed, is derived from neural crest cells originating in the hindbrain (Le Douarin, 1982).

* Data for *Hox-6.1* are from Sharpe *et al.* (1988) and, for the thyroid, from further examination of the autoradiograms prepared by Sharpe *et al.* (1988). As shown for *Hox-3.1* (Fig. 6C), *Hox-6.1* transcripts were not detected in the pharyngeal region.

Table 1 compares the transcript patterns now demonstrated in organs of midgestation mouse embryos with that shown by another mouse homeobox gene, *Hox-6.1* (Sharpe *et al.* 1988). It is seen that the transcript domain for *Hox-6.1* differs from, but overlaps with, the transcript domains for *Hox-1.5* and *Hox-3.1*. Presented in the form shown in Table 1, the data suggest that it is the position of an organ along the anteroposterior axis that determines the range of homeobox gene transcripts present. This seems reasonable for *Hox-3.1* and *Hox-1.5* since it is position along the anteroposterior axis of 7½- to 8¼-day embryos that determines presence or absence of *Hox-3.1* and *Hox-1.5* transcripts. It is important to note, however, that the organs listed in Table 1 may also differ in their positions of origin along the lateral axis of the embryo. Position along lateral, or even dorsoventral, axes might possibly be additional factors that influence the expression of some homeobox genes. Thus, it is not clear at the present time that all homeobox genes and organs can be expected to conform to the simple hierarchical pattern shown in Table 1. Our recent additional observations on the distributions of *Hox-1.2*, *1.3* and *1.4* transcripts in 12½-day embryos do conform to the pattern of Table 1

Fig. 6. Expression of *Hox-3.1* and *Hox-1.5* in the 12½-day embryo. All sections are from the same embryo, and are either near-sagittal (Fig. 6A) or parasagittal (Fig. 6B). Areas shown under dark-field illumination (Fig. 6C–H) are outlined on the corresponding bright-field views (Fig. 6A,B). *Hox-3.1* (Fig. 6C,E,G) and *Hox-1.5* (Fig. 6D,F,H) are compared on adjacent or nearby sections. *tel*, telencephalon; *my*, myelencephalon; *t*, tongue; *p*, pharynx; *pv1*, *pv12*, prevertebrae 1 and 12; *thd*, thyroid duct; *thg*, thyroid gland; *sc*, spinal cord; *ln*, lung; *all*, allantoic stem; *int*, intestine; *h*, heart; *lv*, liver; *st*, stomach; *mes*, mesonephric kidney; *met*, metanephric kidney. Bar, 0·5 mm. Within central nervous tissue, anterior limits of the transcription domains for *Hox-3.1* and *Hox-1.5* are indicated by large arrows on Fig. 6A. Within prevertebral tissue, anterior limits of transcription domains were at the twelfth prevertebra for *Hox-3.1* (Fig. 6E), and anterior to the first prevertebra for *Hox-1.5* (Fig. 6D). The thyroid gland and floor of the pharynx, the lung and the stomach were negative for *Hox-3.1* transcripts (Fig. 6C,E,G) but positive for *Hox-1.5* transcripts (Fig. 6D,F,H). The mesonephric and metanephric kidneys were positive for both *Hox-3.1* (Fig. 6G) and *Hox-1.5* transcripts (Fig. 6H).

(S. J. Gaunt, P. T. Sharpe & D. Duboule, unpublished data). However, *Hox-2.1* does not conform closely, since expression is found in the lung, stomach and metanephric kidney but is weak or absent in prevertebrae (Holland & Hogan, 1988). It is of interest to note that the mesenchymal component of the thyroid gland, unlike that of the other organs described, is derived from neural crest cells (Le Douarin, 1982). Holland & Hogan (1988) previously observed *Hox-2.1* expression in autonomic ganglia derived from the neural crest.

The transcription patterns of *Drosophila* homeotic genes show two important points of similarity with those now described for homeobox genes of the mouse. First, the transcription domains are established early within the germ layers (ectoderm and mesoderm) of the developing *Drosophila* embryo (Levine *et al.* 1983; Akam & Martinez-Arias, 1985) with vestiges of this original pattern remaining in descendant structures, such as the imaginal discs of third instar larvae (Akam, 1983; Wirz *et al.* 1986). Second, the transcripts of most *Drosophila* homeotic genes occupy different, but overlapping, spatial domains. This is seen most clearly for *Antennapedia* and for genes of the Bithorax complex (BX-C) (e.g. Harding *et al.* 1985). It is an interesting possibility, therefore, that homeobox genes might regulate cellular determination along the anteroposterior axis of the mouse by similar mechanisms to those proposed by Lewis (1978) for genes in the BX-C of *Drosophila* (compare Table 1 of this paper with fig. 4 of Lawrence & Morata, 1983). In Lewis's model, and its interpretion by Lawrence & Morata (1983), each gene of the BX-C plays its main role in that segment where it is expressed most anteriorly, but it may also play a lesser role in more posterior segments. Thus, it is the total array of homeotic genes active in a particular group of cells which may determine the final phenotype. In Lewis's model, the BX-C gene products within each segment of *Drosophila* promote the development of structures appropriate to that segment and suppress structures characteristic of more anterior parts. Holland & Hogan (1988) have previously suggested that mouse homeobox genes, like homeotic genes of *Drosophila*, might act combinatorially to effect positional specification.

If mouse homeobox genes provide positional cues for the specification of tissue determination during embryogenesis (Gaunt, 1987), then the patterns of homeobox gene expression now observed at 7½ and 12½ days may be directly linked by cell lineage. The scheme of homeobox gene activity presented as Table 1 might thus be used to construct a fate map for the germ-layer-stage embryo. Such a conclusion is implicit in the earlier work of Dony & Gruss (1987) and Holland & Hogan (1988). Two points of caution should be considered in any comparison of the patterns of homeobox gene expression at early and later stages of embryogenesis. First, expression at 7½ days may be subject to later modification associated with the focusing of transcript boundaries (Gaunt, 1987). Second, many organs begin to cease expression of homeobox genes in the second half of gestation (Dony & Gruss, 1987).

Some aspects of vertebrate development, especially of development within the mesoderm, clearly occur in an anterior-to-posterior sequence. Anterior mesoderm, for example, precedes posterior mesoderm both in times of movement away from the primitive streak and of segmentation to somites. Thus, somite 1 (labelled by the *Hox-1.5* probe) forms at about 8 days, while somites 16 and 17 (suggested by Holland & Hogan, 1988, to be the precursors of prevertebra 12, and thus probably the first to be labelled by the *Hox-3.1* probe) form at 9–9½ days (Rugh, 1968). It is now demonstrated that both *Hox-1.5* and *Hox-3.1* transcripts are first detected by *in situ* hybridization at about 7½ days, but with *Hox-3.1* transcripts apparently following *Hox-1.5* after a small delay. These data show clearly that the time course over which homeobox genes are first expressed does not correspond to the time course of somite formation. Unfortunately, the data now presented, and our knowledge of cell behaviour in the 7½-day embryo, are too imprecise to indicate whether or not activation of homeobox genes corresponds in its time course with that of mesoderm invagination through the primitive streak. In one popular hypothesis (discussed by Gaunt, 1987) it is during invagination through the primitive streak that mesoderm cells first acquire their positional address; ectoderm cells then acquire positional information secondarily by induction from the underlying mesoderm. The data obtained so far are probably most consistent with an alternative model in which all homeobox genes are activated as a single event, but with a slight anterior-to-posterior delay between different genes. This event would commence at about the time that mesoderm completes its migration to the anterior tip of the embryo. Such a model is in-keeping with the finding from Northern blotting experiments that several different homeobox genes of *Xenopus* are activated in late gastrulae (Carrasco *et al.* 1984; Harvey *et al.* 1986; Condie & Harland, 1987).

Although the body plan of the mouse might be specified by the spatial patterns of homeobox gene expression first established at about 7½ days, and then subsequently focused in their transcript boundaries (Gaunt, 1987), the important question remains as to what is the underlying mechanism by which this early pattern is generated. In *Drosophila*, the spatial patterns of homeotic gene expression probably arise,

in a complex series of steps (reviewed by Akam, 1987), from at least two gradients of morphogenetic material which are already present in the egg (Frohnhöfer & Nüsslein-Volhard, 1986; Lehmann & Nüsslein-Volhard, 1986). In the mouse embryo, however, there are probably no such anteroposterior determinants until first appearance of the primitive streak (discussed by Gaunt, 1987). Thus, the mouse embryo may differ from the fly embryo in that it must generate *de novo* during gastrulation a gradient of positional information. Two alternative mechanisms might account for this. In a 'cascade model', the product of one homeobox gene might, after an interval required for its accumulation, activate the next homeobox gene along the anterior-to-posterior sequence. If activation occurred in migrating mesoderm cells, but only during the period of time that they remained within the vicinity of the primitive streak, then the overall pattern of gene activity generated along the anteroposterior axis of the embryo would resemble that shown in Table 1. Breier *et al.* (1986) have already suggested some essential features of a cascade model. In an alternative 'morphogen gradient model', a gradient of morphogen within the epiblast or newly formed germ layers might underlie the generation of positional information. In this model, homeobox genes would differ in the thresholds of morphogen concentration above which they became activated. Retinoic acid, a likely morphogen in the chick limb bud (Thaller & Eichele, 1987), is one candidate for possible involvement. This substance induces homeobox gene activity in mouse teratocarcinoma cells (e.g. Breier *et al.* 1986), but by a mechanism which may not simply be a consequence of its effect upon differentiation (Deschamps *et al.* 1987).

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