Development 104 Supplement, 169–179 (1988)
Printed in Great Britain © The Company of Biologists Limited 1988

Spatially restricted domains of homeo-gene transcripts in mouse embryos: relation to a segmented body plan

STEPHEN J. GAUNT[1], PAUL T. SHARPE[2] and DENIS DUBOULE[3,*]

[1]*Department of Molecular Embryology, AFRC Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT, UK*
[2]*Department of Cell and Structural Biology, Stopford Building, University of Manchester, Manchester M13 9PT, UK*
[3]*Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique de l'INSERM, Faculté de Médecine, 11 rue Humann, 67085 Strasbourg Cédex France*

**Present address*: EMBL, Postfach 10.22 09, D-6900 Heidelberg, FRG

Summary

By use of *in situ* hybridization experiments, the transcripts of several different mouse homeo-genes (*Hox-1.2*, *-1.3*, *-1.4*, *-1.5*, *-3.1* and *-6.1*) have been localized in 12½-day mouse embryos. In a comparison of these genes on adjacent or nearby embryo sections, it is found that their transcripts occupy domains which are usually different, although overlapping, along the anteroposterior axis of the body. The domains are not limited to single segments (assumed to be represented by single prevertebrae) but they encompass regions of adjacent segments. In addition to the prevertebral column, the transcript domains extend into the central nervous system and at least some of the organs (pharynx, thyroid, trachea, lung, stomach and kidney).

Within the prevertebral column, a striking feature of most of the domains is that the abundance of transcripts rises (anteriorly) and falls (posteriorly) over a distance of several adjacent prevertebrae. For *Hox-1.4* and *Hox-1.3* the rise is over prevertebrae within the cervical region. For *Hox-6.1*, *Hox-1·2* and *Hox-3.1*, the rise is over prevertebrae within the thoracic region. For each of the genes examined, transcripts in the central nervous system extend to a more anterior position in the body than transcripts in the prevertebral column. The myelencephalon of the hindbrain contains at least three different anterior boundaries for homeo-gene transcript domains. The positions of these are defined by *Hox-1.5* (most anterior), *Hox-1.4* and *Hox-1.3*. Anterior boundaries for *Hox-6.1* and *Hox-1.2* are apparently located at the *Hox-1.3* position. Homeo-gene transcript domains extend into several structures known to be derived, at least in part, from the neural crest. These include the ventral pharynx, thyroid, aortic trunk and, probably, the sympathetic nerve chain and thymus.

For several genes of the Hox-1 cluster, we note a correspondence between the serial arrangement of genes on the chromosome and the arrangement of their transcript domains in the developing embryo. We also note some striking similarities between the transcript domains of different homeo-genes that share the same subfamily (Duboule *et al.* 1988). These observations, and others, offer possible clues about the molecular mechanisms that might underlie the formation and maintenance of homeo-gene transcript domains.

Key words: mouse embryo, homeo-genes, *in situ* hybridization, transcript domains, prevertebrae, organs, segmentation.

Introduction

The homeobox-containing genes (homeo-genes) of *Drosophila* play a central role in pattern formation during embryogenesis. They participate both in the establishment of segmentation and in the specification of segment identity (e.g. Gehring, 1987). *In situ* hybridization experiments on *Drosophila* em-

bryos have shown that homeo-genes are expressed in a segmentally restricted manner and that their expression presages morphological differentiation of segmental structures (e.g. Levine *et al.* 1983; Akam & Martinez-Arias, 1985; Chadwick & McGinnis, 1987). The discovery of homeo-genes in a wide variety of metazoa, including vertebrates, led to the suggestion that these genes might play a more universal role in pattern formation within the animal kingdom (McGinnis *et al.* 1984). In species that are segmented, this role might, as in *Drosophila*, be intimately linked to segmentation (Struhl, 1984; Ruddle *et al.* 1985). The mouse is one such segmented species (e.g. Hogan *et al.* 1985) since its body is made up, at least in part, of serially repeating structures such as somites, vertebrae, ribs, muscles and nerves.

In situ hybridization studies using several different mouse homeo-genes have shown that their transcripts occupy domains which are spatially restricted along the anteroposterior axis of the developing embryo (Awgulewitsch *et al.* 1986; Gaunt *et al.* 1986; Krumlauf *et al.* 1987; Utset *et al.* 1987; Dony & Gruss, 1987; Toth *et al.* 1987; Gaunt, 1987, 1988; Holland & Hogan, 1988; Sharpe *et al.* 1988). These transcript domains are first established within the ectoderm and mesoderm germ layers at 7½ to 8 days gestation (results for *Hox-1.5* and *Hox-3.1*, Gaunt, 1988), but subsequently they persist within the developing nervous system, the prevertebral column, and within at least some of the organs at 12½ days (e.g. Dony & Gruss, 1987; Holland & Hogan, 1988; Sharpe *et al.* 1988; Gaunt, 1988).

In attempt to obtain directly comparable data on the position of the transcript domains for several different mouse homeo-genes (*Hox-1.2*, *-1.3*, *-1.4*, *-6.1* and *-3.1*) we have now localized their transcripts by *in situ* hybridization to nearby sections of the same 12½-day embryo. For additional comparison, we also present results for *Hox-1.5*. The location of the transcript domains is described in relation to the sequence of segments (seen principally in the sequence of prevertebrae) along the body axis.

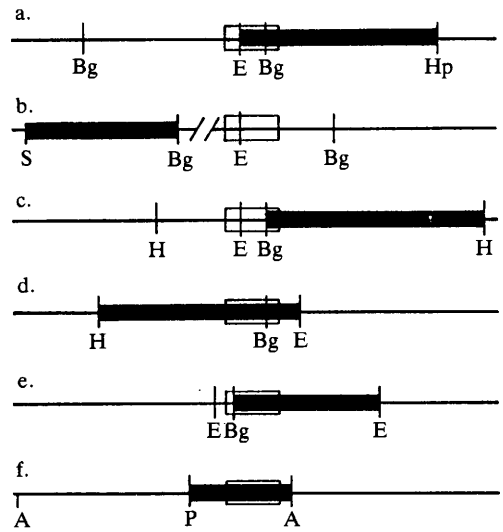

Fig. 1. Probes used (solid bars) in the *in situ* hybridization experiments. a, *Hox-1.2*; b, *Hox-1.3*; c, *Hox-1.4*; d, *Hox-1.5*; e, *Hox-6.1*; f, *Hox-3.1*. Fragments for use as probes were cut from genomic or cDNA (*Hox-6.1*) clones, and then subcloned into Gemini transcription vectors (Promega). Open boxes are the homeoboxes. E, *Eco*RI; Bg, *Bgl*II; Hp, *Hpa*II; S, *Sac*I; H, *Hin*dIII; A, *Ava*I; P, *Pst*I. More complete restriction maps for these genes are provided by Duboule *et al.* (1986, for *Hox-1.2*, *Hox-1.4*, *Hox-1.5*), Fibi *et al.* (1988, for *Hox-1.3*), Sharpe *et al.* (1988, for *Hox-6.1*) and Breier *et al.* (1986, for *Hox-3.1*). For each of the genes shown, normal transcription is from left to right. For use in *in situ* hybridization, ^{35}S-labelled antisense RNA probes were synthesized in the opposite direction, as described by Gaunt *et al.* (1986) and Gaunt (1987). Alkaline hydrolysis of labelled probes and use in *in situ* hybridization to embryo sections (7 μm thick) were as previously described (Gaunt *et al.* 1986; Gaunt, 1987).

Transcript domains in the prevertebral column

The transcript domains in the prevertebral column for *Hox-1.4*, *-1.3*, *-6.1*, *-1.2* and *-3.1*, detected by *in situ* hybridization of ^{35}S-labelled RNA probes (Fig. 1) to nearby sections of the same 12½-day embryo (Fig. 2), are shown in Fig. 3. The description now given is based not only upon Fig. 3, but also upon observations made in other sections from the same, and from two additional, 12½-day embryos. Unless otherwise indicated, the transcript domain for each gene apparently remained the same at all positions across the lateral axis of the prevertebral column. For all genes, anterior parts of thoracic vertebrae (see legend to Fig. 2) were found to be labelled more intensely than corresponding posterior parts.

Hox-1.4 transcripts (Fig. 3A) were most abundant in the cervical region. No labelling above background was found in prevertebra 1 (pv1). Pv2 was weakly labelled, and pv3–7 were strongly labelled. Labelling intensity progressively, and markedly, declined over the first four thoracic vertebrae (pv8–11). This decline, seen at all positions across the lateral axis of the prevertebral column, was not a plane-of-section artefact. Labelling intensity continued to decline progressively in more posterior positions but remained above background at least until pv18.

For *Hox-1.3* (Fig. 3B), transcripts were not detected (or were at most barely detected above background in only a few sections) in pv2. Labelling was weak in pv3, but progressively increased in intensity over pv3–6. Pv6 and 7 were intensely

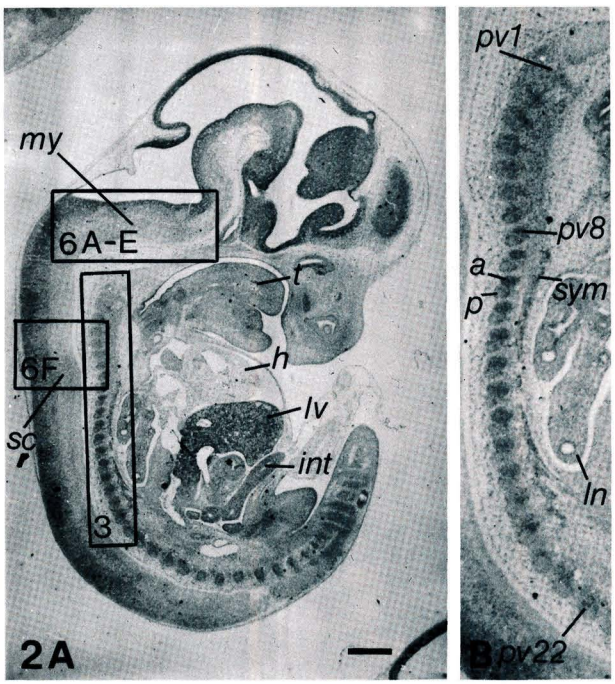

Fig. 2. Parasagittal section of the 12½-day mouse embryo, viewed by bright-field illumination, to illustrate the areas examined for homeo-gene transcripts in Figs 3, 6A–E and F. B is an enlargement of the prevertebral column shown in A. *my*, myelencephalon; *sc*, spinal cord; *int*, intestine; *lv*, liver; *h*, heart; *t*, tongue; *pv1*, *pv8*, *pv22*, prevertebrae 1, 8 and 22; *a*, *p*, anterior and posterior parts of thoracic vertebrae; *ln*, lung. Bar, 0·5 mm. At present, our identification of the sympathetic nerve chain (*sym*) is tentative. During examination of autoradiograms, position along the prevertebral column was sometimes identifiable by counting backwards from pv1. In many sections, however, pv1 was difficult to identify and an alternative, more reliable, landmark was found in pv8 (the first thoracic prevertebra). In parasagittal sections (such as shown here) the thoracic prevertebrae characteristically comprise anterior and posterior parts (Fig. 2B). The anterior part may be related to the rib, although we have not yet confirmed this.

labelled. More posteriorly, intensity of *Hox-1.3* labelling was progressively reduced. In some sections (as found for *Hox-1.4*), labelling intensity declined substantially over the first few thoracic vertebrae. In other sections, and as shown in Fig. 3B, the anterior-to-posterior decline over thoracic vertebrae was clearly more gradual than was seen for *Hox-1.4*. Labelling for *Hox-1.3* remained above background at least until pv24.

Hox-6.1 transcripts were most abundant in the thoracic region (Fig. 3C). Pv1–6 were not labelled above background. Pv7 was weakly labelled in a few sections, but pv8 was often the first prevertebra seen to be labelled. Labelling increased in intensity over pv8–12. Pv12–16 were the prevertebrae most intensely labelled. More posteriorly, labelling intensity progressively declined.

In some sections, the distribution of *Hox-1.2* transcripts within the prevertebral column appeared to be identical to that described for *Hox-6.1*. However, *Hox-1.2* transcripts were never detected in pv7. Furthermore, in several sections (such as shown in Fig. 3D) the fall in abundance of *Hox-1.2* transcripts posterior to pv16 was more gradual than that seen for *Hox-6.1*.

For *Hox-3.1* (Fig. 3E), pv12 was usually the most anterior position for detection of transcripts. In some sections, however, weak labelling above background was seen in pv11. Labelling was intense over pv13–16 and was then progressively reduced over more posterior prevertebrae.

The results now presented can be compared with *in situ* hybridization data presented previously. For *Hox-1.5*, the transcript domain within the prevertebral column extends to a more anterior position than do the domains described above. Thus, *Hox-1.5* transcripts are detected strongly in pv1 but, as now described for other genes, abundance of transcripts declines in more posterior parts of the prevertebral column (see figures provided by Gaunt, 1988). Results similar or identical to those now described have already been published for *Hox-6.1* (Sharpe *et al.* 1988), *Hox-1.2* (Toth *et al.* 1987) and *Hox-3.1* (Holland & Hogan, 1988; Gaunt, 1988). However, our results differ from those of Dony & Gruss (1987) who reported pv8 as the most anterior position for detection of *Hox-1.3* transcripts.

Transcript domains in pharyngeal, thoracic and abdominal organs

Fig. 4A shows the arrangement of tissues in the vicinity of the pharynx, trachea and lung. In midsagittal sections, three separate ducts were seen to lead from the pharynx: the thyroid duct, the trachea and the oesophagus. A structure that we have identified as the thyroid gland lies at the base of the thyroid duct, deep in the pharyngeal floor (see Rugh, 1968). Fig. 4B–E shows the distributions of *Hox-1.4*, *Hox-1.2*, *Hox-1.5* and *Hox-3.1* transcripts as detected mainly within the mesodermal components of these tissues.

For *Hox-1.5*, the pattern of labelling observed within pharyngeal tissues was as previously described (Gaunt, 1988). Thus, *Hox-1.5* transcripts were detected in most of the tissues that formed the floor of the pharynx, including the thyroid gland (Figs 4D and 5). Labelling given by the *Hox-1.5* probe extended anterior to the thyroid duct, but did not extend over

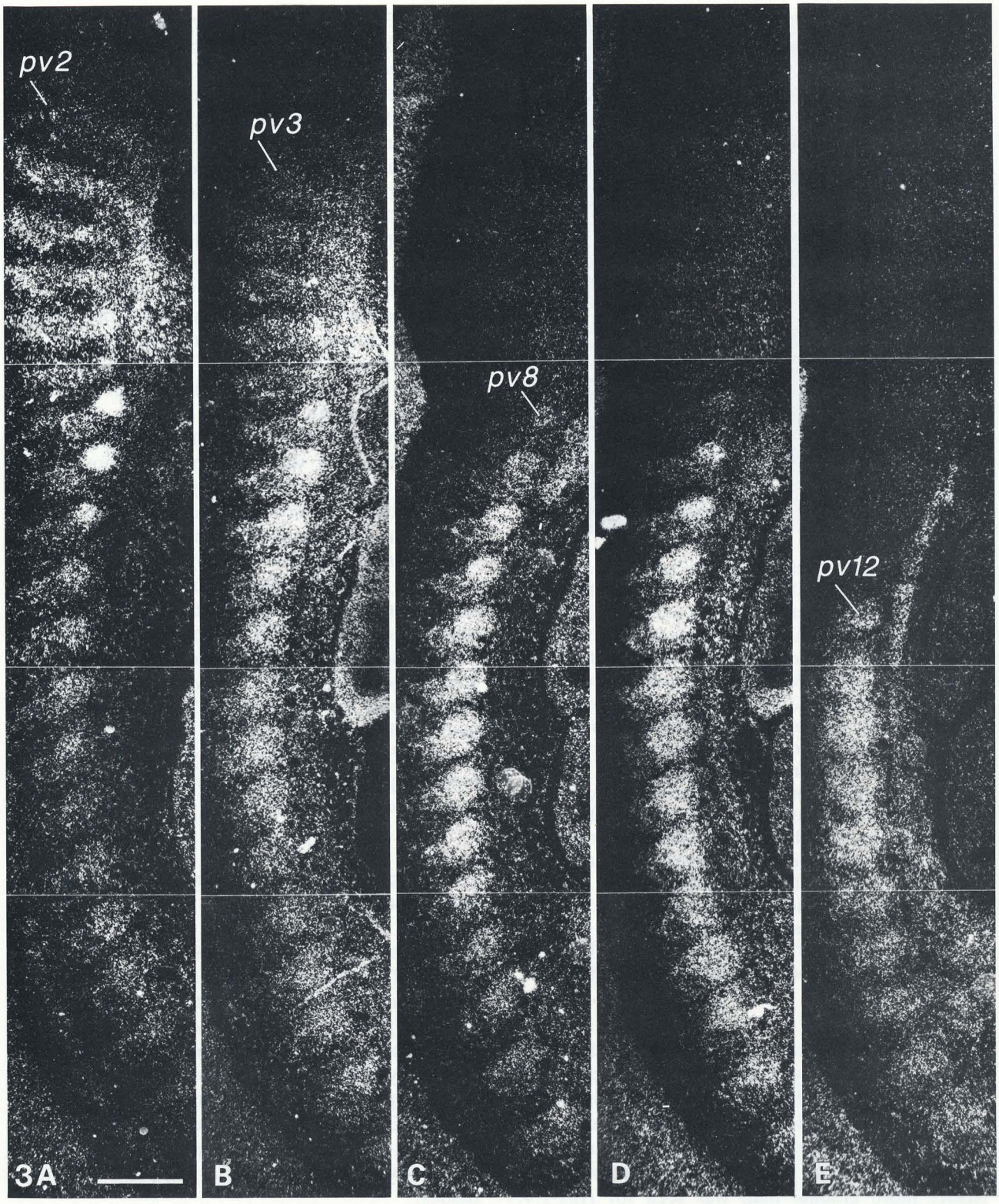

Fig. 3. Homeo-gene transcript domains localized by *in situ* hybridization within the prevertebral column. (A) *Hox-1.4*; (B) *Hox-1.3*; (C) *Hox-6.1*; (D) *Hox-1.2*; (E) *Hox-3.1*. The sections are parasagittal, and are cut from the same embryo. The sequence, and therefore proximity, of the sections shown is: 1st section (A), 8th (B), 4th (C), 9th (D), 13th (E). All sections are viewed under dark-field illumination. A bright-field view of section E is shown in Fig. 2B. *pv2*, *pv3*, *pv8*, *pv12*, prevertebrae 2, 3, 8 and 12. Bar, 0·2 mm.

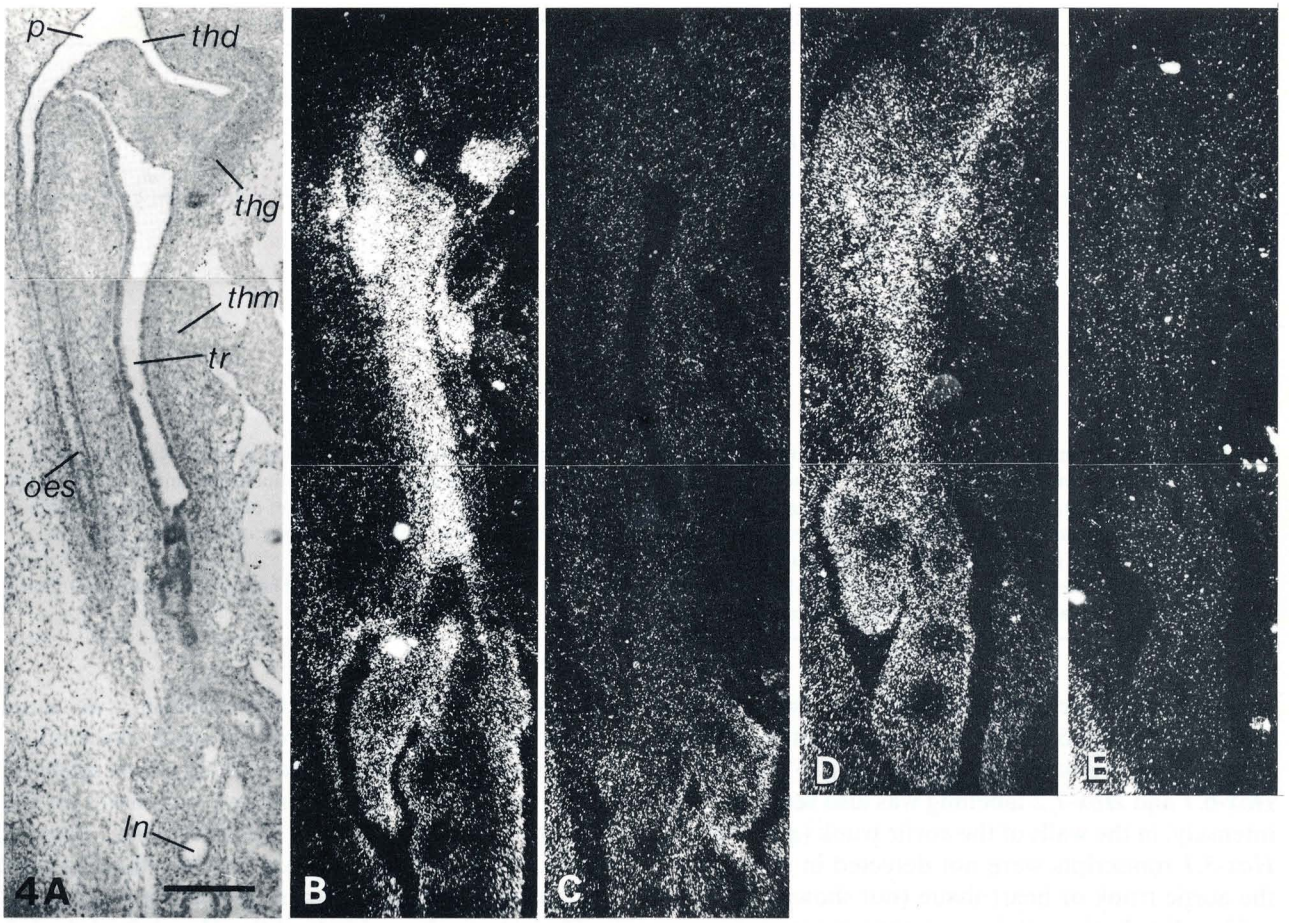

Fig. 4. *In situ* hybridization to localize homeo-gene transcripts within tissues that surround the pharynx, trachea and lung. (A) bright-field, (B–E) dark-field illumination. (B) *Hox-1.4*; (C) *Hox-1.2*; (D) *Hox-1.5*; (E) *Hox-3.1*. A–C are nearby sections from the same 12½-day embryo. D and E are nearby sections from a second, slightly smaller, 12½-day embryo. *p*, pharynx; *thd*, thyroid duct; *thg*, thyroid gland; *oes*, oesophagus; *tr*, trachea; *ln*, lung. Bar, 0·2 mm. At present, our identification of the thymus (*thm*) is tentative.

the tongue (Fig. 5). Posterior to the pharynx, *Hox-1.5* transcripts were readily detected in the trachea and the lung (Fig. 4D). *Hox-1.4* transcripts were detected in the thyroid gland but, unlike *Hox-1.5* transcripts, were not present generally in tissues that formed the floor of the pharynx (Fig. 4B). *Hox-1.4* transcripts were not, therefore, detectable anterior to the thyroid duct. *Hox-1.4* transcripts were abundant in the trachea and lung (Fig. 4B). The distribution of *Hox-1.3* transcripts throughout these tissues (not shown) was similar to that now described for *Hox-1.4*. *Hox-1.2* transcripts (Fig. 4C) were not detected in any pharyngeal tissue, including the thyroid gland, or the trachea. *Hox-1.2* transcripts were, however, readily detected in the lung. A similar result was given by the *Hox-6.1* probe, although weak labelling within the trachea, only slightly higher than the background level, was noted for this probe (not shown). For all four of these genes, the pattern of labelling within the lung seemed to be identical. Thus, labelling was restricted to mesodermal components and did not include the endodermally derived lining epithelium. Sections that passed through the lumen of the trachea (sections hybridized to the *Hox-1.3* probe and not shown here) were similarly labelled within mesodermal but not endodermal (lining epithelial) components. *Hox-3.1* transcripts were not detected in any part of the pharynx, trachea or lung (Fig. 4E).

These findings are summarized in Table 1, together with results for the heart, stomach, mesonephric and metanephric kidneys. For all six genes studied, transcripts were readily detected in mesonephric and metanephric kidneys but without localization to any particular region (not shown, but see Gaunt, 1988, for distribution of *Hox-1.5* and *Hox-3.1* transcripts). Within the stomach, homeo-gene transcripts were restricted to the mesodermal components and were not evident within the endodermally derived lining epithelium (not shown; see Gaunt, 1988).

Although heart tissue has been scored negative for all transcripts in Table 1, *Hox-1.5* and *Hox-1.4* tran-

Table 1. *Homeo-gene expression in organs of midgestation embryos*

	Homeo-gene (anteriormost prevertebrae showing expression)					
	Hox-1.5 (1)	*Hox-1.4* (2–3)*	*Hox-1.3* (3–6)	*Hox-6.1* (7–12)	*Hox-1.2* (8–12)	*Hox-3.1* (11–13)
Heart	–	–	–	–	–	–
Ventral pharynx	+	–	–	–	–	–
Thyroid	+	+	+	–	–	–
Trachea	+	+	+	–	–	–
Lung	+	+	+	+	+	–
Stomach	+	+	+	+	+	–
Mesonephric kidney	+	+	+	+	+	+
Metanephric kidney	+	+	+	+	+	+

The heart, lung, stomach, mesonephric and metanephric kidneys are listed in a sequence that 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 (Holland & Hogan, 1988; Gaunt, 1988). The mesodermal origin of the trachea is assumed to lie anterior to the origin of the lung, but posterior to the origin of the heart. The mesodermal component of the thyroid and ventral pharynx, unlike that of the other organs listed, is probably derived from neural crest cells that originate in the hindbrain (Le Douarin, 1982).

*Numbers given refer to the prevertebrae over which there is an increase in abundance of transcripts. These prevertebrae therefore lie at the anterior boundary of the transcript domain.

scripts were detected both within the walls of the aortic trunk (Fig. 5) and within a small patch of tissue restricted to the base of the heart (Fig. 5). *Hox-1.3*, *Hox-6.1* and *Hox-1.2* labelling was also seen, but less intensely, in the walls of the aortic trunk (not shown). *Hox-3.1* transcripts were not detected in any part of the aortic trunk or heart tissue (not shown).

The distribution of homeo-gene transcripts now observed within the lung, stomach and kidney are as previously described for *Hox-1.3* (Dony & Gruss, 1987), *Hox-2.1* (Holland & Hogan, 1988), *Hox-6.1* (Sharpe *et al.* 1988), *Hox-1.5* and *Hox-3.1* (Gaunt, 1988). The new data now provided in Table 1 expand the table constructed earlier by Gaunt (1988) and, presented in this form, suggest that the position of an organ along the anteroposterior axis is at least one important factor that determines the range of homeo-gene transcripts present.

Transcript domains in the central nervous system

For transcript domains within the central nervous system, as in the prevertebral column, two main observations can be made from *in situ* hybridizations to longitudinal sections of the 12½-day mouse embryo. First, the position of the anterior boundary of the transcript domain can be identified and, second, anterior-to-posterior variation in the abundance of transcripts within the domain can be observed.

The anterior boundaries of the *Hox-1.5*, *-1.4*, *-1.3*,

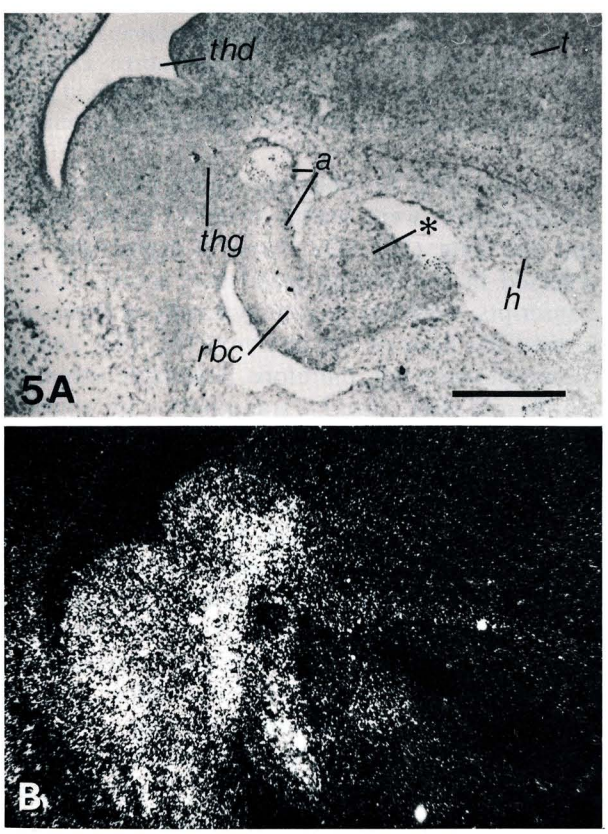

Fig. 5. *Hox-1.5* transcripts detected by *in situ* hybridization within the wall of the aortic trunk. (A) bright-field, (B) dark-field illumination. *a*, aortic trunk seen in both transverse and longitudinal sections; *rbc*, red blood cells; *h*, heart tissue; *, patch of labelled tissue at the base of the heart; *t*, tongue; *thd*, thyroid duct; *thg*, thyroid gland. Bar, 0·2 mm. The exact boundary between the aortic trunk and the heart tissue (bulbous arteriosus region) is uncertain. rbc appear bright under dark-field illumination. This is a property of the rbc tissue itself, and is not due to overlying silver grains.

-6.1, *-1.2* and *-3.1* transcript domains are shown in Fig. 6. We observed four distinctly different positions for the boundary. First, for *Hox-1.5*, the boundary lay in the anterior part of the floor of the myelencephalon (Fig. 6A). Second, for *Hox-1.4*, the boundary lay about midway along the floor of the myelencephalon (Fig. 6B). Third, for *Hox-1.3*, *Hox-6.1* and *Hox-1.2*, the boundary lay in apparently the same position, in the posterior floor of the myelencephalon (Fig. 6C–E). Fourth, for *Hox-3.1*, the boundary lay within the spinal cord and was at the level of the 5th prevertebra in the ventral part of the cord and the 3rd prevertebra in the middle of the cord (Fig. 6F). Fig. 6 shows clearly that the anterior boundaries of the transcript domains are irregular in shape along the dorsoventral axis of the nervous tissue. For each gene, however, these irregularities in shape have been found to be consistent between different embryos (not shown). Furthermore, the shape of the boundaries for *Hox-1.3*, *Hox-6.1* and *Hox-1.2* (Fig. 6C–E) are, in addition to their anteroposterior position, apparently the same. The data shown in Fig. 6 were obtained from one region across the lateral axis of the central nervous system (close, but lateral, to the central canal of the spinal cord). It is important to note that we cannot, at the present time, be certain that *Hox-1.3*, *Hox-6.1* and *Hox-1.2* transcripts share the same anterior boundary at all positions across the lateral axis of the central nervous system.

These findings are consistent with results published earlier for *Hox-1.5* (Gaunt, 1987; Fainsod *et al.* 1987), *Hox-1.3* (Dony & Gruss, 1987), *Hox-1.4* and *Hox-1.2* (Toth *et al.* 1987), *Hox-6.1* (Sharpe *et al.* 1988) and *Hox-3.1* (Awgulewitsch *et al.* 1986; Utset *et al.* 1987; Holland & Hogan, 1988). However, the new data, obtained on nearby sections from the same embryo, are an advance on earlier results since they demonstrate at least three different anterior boundaries for homeo-gene transcript domains within the myelencephalon. It is possible (as in our earlier interpretation for *Hox-6.1*, Sharpe *et al.* 1988) that the most posterior of these three boundaries is, in fact, located just behind the myelencephalon. The irregularities in shape of the anterior boundaries may possibly develop, as suggested earlier (Gaunt, 1987), as a result of cell movement within the nervous tissue.

We have not yet made a detailed comparison of

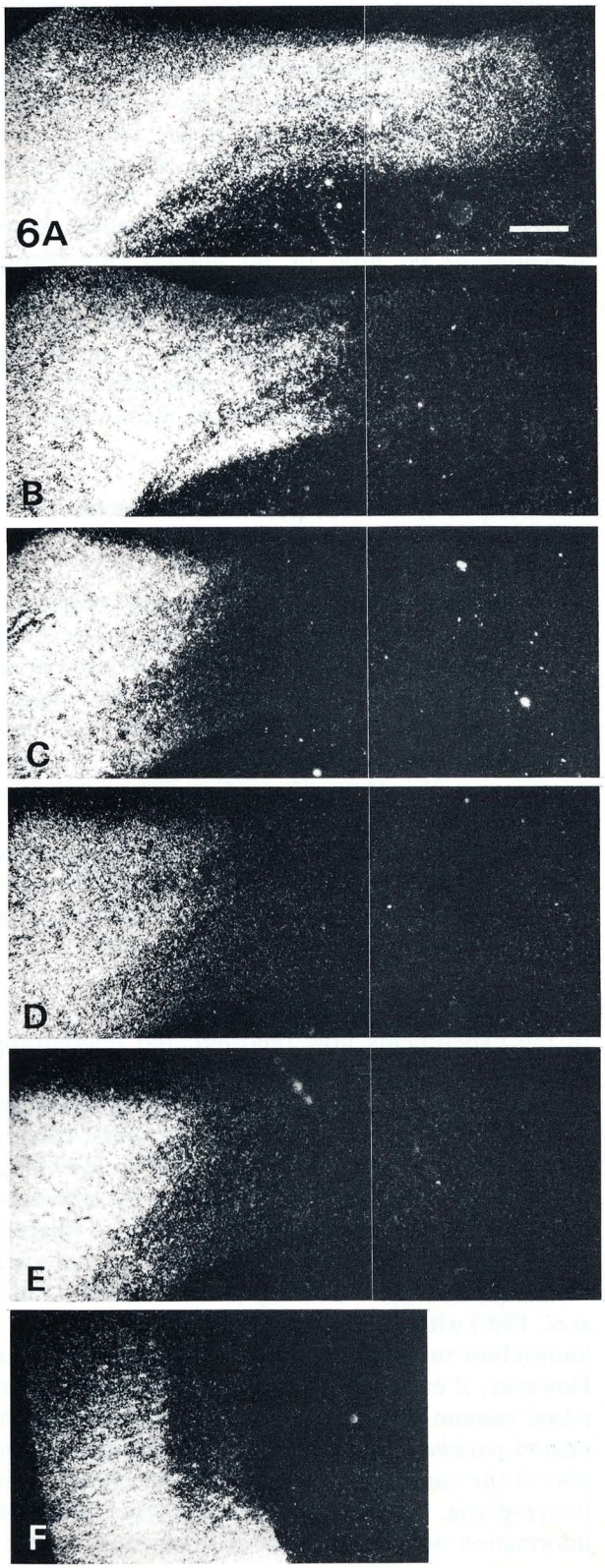

Fig. 6. Anterior boundaries of homeo-gene transcript domains detected by *in situ* hybridization within the central nervous system. The fields shown are outlined on the bright-field view of the whole embryo, Fig. 2A. (A) *Hox-1.5*; (B) *Hox-1.4*; (C) *Hox-1.3*; (D) *Hox-6.1*; (E) *Hox-1.2*; (F) *Hox-3.1*. The sections are parasagittal. Sections B–F were cut from the same embryo. The sequence, and therefore proximity, of the sections is as given under Fig. 3. Section A was cut from a different $12\frac{1}{2}$-day embryo but, in other experiments (not shown), we have compared *Hox-1.5* and *Hox-1.4* on adjacent sections and have observed differences in their transcript boundaries similar to those shown here. All sections are viewed under dark-field illumination. Bar, 0·2 mm.

different homeo-genes with respect to the anterior-to-posterior distribution of their transcripts along the length of the spinal cord. These distributions are complex due to the fact that the abundance of transcripts may vary across the lateral and dorsoventral axes of the spinal cord (Utset *et al.* 1987; Toth *et al.* 1987; Holland & Hogan, 1988), and also possibly due to cell movement within nervous tissue (Gaunt, 1987). We earlier described the progressive anterior-to-posterior fall in abundance of *Hox-1.5* transcripts along the spinal cord (Gaunt *et al.* 1986; Gaunt, 1987). We have now made similar observations for the transcripts of *Hox-1.4*, *Hox-1.3*, *Hox-1.2* and *Hox-3.1* (not shown). For *Hox-6.1*, however, there appeared to be little or no anterior-to-posterior reduction in the abundance of transcripts (Sharpe *et al.* 1988). Thus, although *Hox-6.1* was not distinguishable from *Hox-1.3* and *Hox-1.2* in the anterior limits of its transcripts, a clear difference was seen in transcript distribution along the spinal cord.

Transcript domains as positional cues on a segmented body plan

We suggested earlier that homeo-gene transcripts might serve as positional cues during the development of both ectoderm- and mesoderm-derived structures of the mouse (Gaunt *et al.* 1986). This suggestion is consistent with the known function of these genes in *Drosophila* (e.g. Gehring, 1987). It is also consistent with observations that different mouse homeo-genes display spatially distinct transcript domains, and with the finding that these domains are established early in mouse development (Gaunt, 1988), at about the time of cellular determination along the anteroposterior axis (Gaunt, 1987).

Domains in mesoderm derivatives

In the experiments now reported, the relationship between homeo-gene transcript domains and body segments is seen most clearly within mesodermal derivatives that give rise to the prevertebral column (Fig. 3). The domains are not limited to individual segments (assumed to be represented by individual prevertebrae) but they encompass regions of adjacent prevertebrae. It seems likely, therefore, that each homeo-gene might exert its positional effect (Gaunt *et al.* 1986) within several adjacent segments. It is not known how such a positional effect might be exerted. However, if each cell within the developing prevertebral column is responsive in its choice of developmental pathway both to the level of abundance and also to the variety of homeo-gene transcripts within its cytoplasm, then it is possible that there is sufficient information in the pattern of transcripts already shown (Fig. 3) to specify position for all cervical and anterior thoracic prevertebrae.

The pattern of homeo-gene transcripts that we have now observed within the mesodermal components of several different organs (Table 1; Gaunt, 1988) might provide, at least in part, the molecular basis for tissue specification during organogenesis. The similarity of this hierarchical pattern to the pattern of expression displayed by homeotic genes in the Bithorax complex of *Drosophila* (Lewis, 1978; Lawrence & Morata, 1983) perhaps suggests that similar mechanisms exist in both flies and vertebrates to specify determination of tissues along the body axis (see Gaunt, 1988). A possible role for homeo-genes in the control of mouse organogenesis has also been discussed by Dony & Gruss (1987) and Holland & Hogan (1988). These authors have pointed out the importance of mesoderm as the instructional component in the development of organs by epithelial–mesenchymal interaction.

Segmentation of the mesoderm germ layer is incomplete in mammals. The somitic mesoderm (which gives rise to vertebrae, ribs, muscles and dermis) and the nephrotomes (which give rise to kidney tissue) are segmented, but the more laterally positioned 'lateral plate mesoderm' (which probably contributes, for example, to the trachea, lung and stomach) is not (e.g. Hogan *et al.* 1985). If an assumption is made that the transcript domain of a homeo-gene occupies a similar anteroposterior position within both segmented and unsegmented mesoderm, then conclusions can be drawn from Table 1 about the location within lateral plate mesoderm of the origins of some thoracic and abdominal organs. The lung and stomach, for example, are apparently formed from mesoderm located anterior to the origins of prevertebrae 11–12 (pv11–12) (since *Hox-3.1* expression, which is not seen in the lung or stomach, is detected in the prevertebral column posterior to pv11–12) but posterior to pv8 (since *Hox-1.2*, which is expressed in the lung and stomach, is not expressed anterior to pv8). Similarly, the trachea apparently develops from mesoderm adjacent to the origins of pv3–7, and the kidney develops from mesoderm posterior to pv12. Homeo-gene transcript data might thus be used to construct a fate map for the germ-layer-stage embryo (Gaunt, 1988). It should be noted that these estimates for the sites of origin in the mouse of the stomach, lung and kidney are widely different from estimates based upon analogy with the chick (discussed by Holland & Hogan, 1988). At present, the reasons for this discrepancy are unclear.

Domains in neural crest derivatives

We found *Hox-1.5*, *Hox-1.4* and *Hox-1.3* transcripts within the thyroid gland and the walls of the aortic

trunk. *Hox-1.5* transcripts were also found in the ventral wall of the pharynx. A common feature of these structures is that the mesenchymal component is thought not to be derived from the mesoderm germ layer, but instead from neural crest cells that originate in the myelencephalon (Le Douarin, 1982). *Hox-1.5* transcripts did not extend anteriorly into the tongue, a structure probably derived from neural crest cells that arise anterior to the myelencephalon (Le Douarin, 1982). These observations are consistent with an extension of homeo-gene transcript domains to neural crest cells. In addition to the above tissues, we have also observed homeo-gene transcripts in two structures that we tentatively identify as the sympathetic nerve chain (Fig. 2B; positive for transcripts of all homeo-genes, e.g. Fig. 3E) and the thymus (Fig. 4; positive only for *Hox-1.5*, *Hox-1.4* and *Hox-1.3* transcripts). Sympathetic nervous tissue and the mesenchymal component of the thymus are both neural crest derivatives (Le Douarin, 1982). Holland & Hogan (1988) previously reported expression of *Hox-2.1* in autonomic ganglia derived from the neural crest. We are hesitant to suggest that homeo-gene transcripts may provide positional cues in neural crest cells since the course of differentiation in these cells seems to be specified not simply by their site of origin in the central nervous system, but instead by environmental cues encountered after migration (reviewed by Le Douarin, 1982).

Domains in the central nervous system

For each of the genes examined, transcripts in the central nervous system extended to a more anterior position in the body than did transcripts in mesodermal derivatives. Our finding that transcripts of three different homeo-genes (*Hox-1.3*, *Hox-6.1* and *Hox-1.2*) apparently share the same anterior boundary was unexpected. If valid, the observation suggests two possibilities. First, two different genes (such as *Hox-1.3* and *Hox-1.2*) may show clearly distinct anterior boundaries within mesodermal derivatives, but may share the same anterior boundary within ectodermal derivatives. Second, the precise position of the anterior boundary may not always be so important as a positional cue as is the distribution of transcripts posterior to the boundary (this distribution for *Hox-6.1* was clearly different to that for *Hox-1.2* and *Hox-1.3*). The *Hox-1.5* boundary within the hindbrain corresponds at earlier stages of development to a neuromere constriction (Gaunt *et al.* 1986; Gaunt, 1987, 1988). A neuromere constriction may be a segmental boundary within the nervous system (Hogan *et al.* 1985). We are currently investigating the possibility that the boundaries defined by the *Hox-1.4* and *Hox-1.3* probes correspond to neuromere constrictions located more posteriorly in the hindbrain.

Possible clues on molecular mechanisms that underlie transcript domains

Since prevertebrae (and the somites from which they are formed) are morphologically separate units, we consider it unlikely that there is cell mixing between them. The prevertebral column may therefore be the ideal structure in which to observe homeo-gene transcript domains, uncomplicated by cell migration. Three aspects of the results now presented offer possible clues about the molecular mechanisms that regulate homeo-gene transcript domains.

First, a striking characteristic of each domain is that the abundance of transcripts rises (anteriorly) and falls (posteriorly) over a distance of several adjacent prevertebrae. In some instances, the rise in abundance of one transcript (e.g. *Hox-6.1* or *Hox-1.2*) seems to be almost complementary to the fall in abundance of another (e.g. *Hox-1.4* or *Hox-1.3*). Studies in *Drosophila* have indicated the importance of inhibitory interactions between homeo-genes in order to generate the final pattern of transcripts (Hafen *et al.* 1984; Harding *et al.* 1985; Struhl & White, 1985). We consider that negative or positive interactions between the products and regulatory sequences of different mouse homeo-genes could generate the transcript patterns now observed in the prevertebral column. These mechanisms are not necessarily the same as mechanisms required earlier in development for the initial establishment of domains (Gaunt, 1988).

Second, there is an apparent correspondence between the relative position of genes within the Hox-1 cluster and that of their transcript domains within the mouse embryo (Fig. 7). It is possible that physical linkage of these genes is an essential feature of mechanisms necessary for their interaction. This could, for example, be *cis*-acting regulatory elements. In *Drosophila*, there is a similar correspondence between the relative position of homeo-genes on chromosomal DNA and that of their transcript domains in embryos (Harding *et al.* 1985).

Third, there is similarity between the transcript domains now detected for *Hox-6.1* and *Hox-1.2*. In addition, there is similarity between the transcript domain now described for *Hox-1.4* and that detected earlier using a *Hox-5.1*-specific probe (Featherstone *et al.* 1988). For both *Hox-1.4* and *Hox-5.1* the anterior boundary of transcripts within the prevertebral column is at the level of the second prevertebra. Duboule *et al.* (1988) have assigned *Hox-1.4* and *Hox-5.1* to a common subfamily based upon close

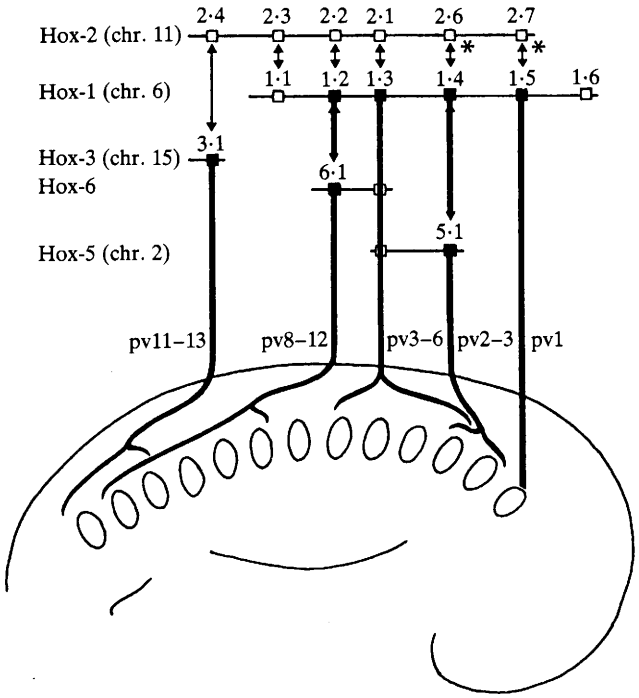

Fig. 7. Summary of homeo-gene transcript patterns in the prevertebral column of the 12½-day mouse embryo. Filled boxes show the homeo-genes that we have examined by *in situ* hybridization. Transcripts may increase in abundance over several adjacent prevertebrae (shown in brackets) at the anterior boundary of each transcript domain. Homeo-genes from different chromosomal clusters are aligned (vertical arrows) in subfamilies (see Duboule *et al.* 1988, for detailed explanation and references; see also Hart *et al.* 1987). Genes within a subfamily show similarities in 'variable' amino acids encoded by the homeobox. 'Variable' amino acids are identified as those which differ from the *Antennapedia* (*Antp*) sequence (Duboule *et al.* 1988). * Denotes personal communication from R. Krumlauf (unpublished data). The *Hox-6.1* homeobox-encoded sequence shows five amino acid changes from that of *Antp*. Of these, three identical changes are seen in either *Hox-1.2* or *Hox-2.2*. At the present time, however, our assignment of *Hox-6.1* to the *Hox-1.2* subfamily remains tentative, awaiting sequence data for neighbouring Hox-6 genes. Recent evidence (K. Schughart and F. H. Ruddle, personal communication) suggests that the original assignment of *Hox-6.1* to chromosome 14 (Sharpe *et al.* 1988) may be incorrect. *Hox-6.1* appears instead to be located on chromosome 15, and might, therefore, be part of the Hox-3 cluster. We continue to use the *Hox-6.1* nomenclature until the precise location is confirmed.

homologies in their amino acid sequence. *Hox-6.1* and *Hox-1.2* might similarly be comembers of a subfamily (see legend to Fig. 7). Thus, our observations so far suggest that homeo-genes within the same subfamily may display similar or identical transcript domains in the developing embryo. The sequences within a homeo-gene that control position of its transcript domain (these sequences are presumed to lie in upstream regions of the gene) might therefore be revealed by analysis of conserved sequence within a subfamily. A more trivial explanation of our results, however, might be cross-reactivity between probes prepared from similar genes. We are currently attempting to distinguish between these two possibilities.

References

Akam, M. E. & Martinez-Arias, A. (1985). The distribution of *Ultrabithorax* transcripts in *Drosophila* embryos. *EMBO J.* **4**, 1689–1700.

Awgulewitsch, A., Utset, M. F., Hart, C. P., McGinnis, W. & Ruddle, F. H. (1986). Spatial restriction in expression of a mouse homeobox locus within the central nervous system. *Nature, Lond.* **320**, 328–335.

Breier, G., Bucan, M., Francke, U., Colberg-Poley, A. M. & Gruss, P. (1986). Sequential expression of murine homeobox genes during F9 EC cell differentiation. *EMBO J.* **5**, 2209–2215.

Chadwick, R. & McGinnis, W. (1987). Temporal and spatial distribution of transcripts from the *Deformed* gene of *Drosophila*. *EMBO J.* **6**, 779–789.

Dony, C. & Gruss, P. (1987). Specific expression of the Hox-1.3 homeobox gene in murine embryonic structures originating from or induced by the mesoderm. *EMBO J.* **6**, 2965–2975.

Duboule, D., Baron, A., Mahl, P. & Galliot, B. (1986). A new homeobox is present in overlapping cosmid clones which define the mouse Hox-1 locus. *EMBO J.* **5**, 1973–1980.

Duboule, D., Galliot, B., Baron, A. & Featherstone, M. S. (1988). Murine homeo-genes: some aspects of their organisation and structure. In *Cell to Cell Signals in Mammalian Development* (ed. S. deLaat, J. G. Bluemink & C. L. Mummery), NATO ASI series, Springer Verlag.

Fainsod, A., Awgulewitsch, A. & Ruddle, F. H. (1987). Expression of the murine homeobox gene Hox-1.5 during embryogenesis. *Devl Biol.* **124**, 125–133.

Featherstone, M. S., Baron, A., Gaunt, S. J., Mattei, M. & Duboule, D. (1988). Hox-5.1 defines a homeo-gene locus on mouse chromosome 2. *Proc. natn. Acad. Sci. U.S.A.* **85**, 4760–4764.

Fibi, M., Zink, B., Kessel, M., Colberg-Poley, A. M., Labeit, S., Lehrach, H. & Gruss, P. (1988). Coding sequence and expression of the homeobox gene Hox-1.3. *Development* **102**, 349–359.

Gaunt, S. J. (1987). Homoeobox gene *Hox-1.5* expression in mouse embryos: earliest detection by *in situ* hybridization is during gastrulation. *Development* **101**, 51–60.

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., MILLER, J. R., POWELL, D. J. & DUBOULE, D. (1986). Homeobox gene expression in mouse embryos varies with position by the primitive streak stage. *Nature, Lond.* **324**, 662–664.

GEHRING, W. J. (1987). Homeoboxes in the study of development. *Science* **236**, 1245–1252.

HAFEN, E., LEVINE, M. & GEHRING, W. J. (1984). Regulation of Antennapedia transcript distribution by the bithorax complex in *Drosophila*. *Nature, Lond.* **307**, 287–289.

HARDING, K., WEDEEN, C., MCGINNIS, W. & LEVINE, M. (1985). Spatially regulated expression of homeotic genes in *Drosophila*. *Science* **229**, 1236–1242.

HART, C. P., FAINSOD, A. & RUDDLE, F. H. (1987). Sequence analysis of the murine Hox-2.2, Hox-2.3 and Hox-2.4 homeo-boxes: evolutionary and structural comparisons. *Genomics* **1**, 182–195.

HOGAN, B., HOLLAND, P. & SCHOFIELD, P. (1985). How is the mouse segmented? *Trends in Genetics* **1**, 67–74.

HOLLAND, P. W. H. & 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.

KRUMLAUF, R., HOLLAND, P. W. H., MCVEY, J. H. & HOGAN, B. L. M. (1987). Developmental and spatial patterns of expression of the mouse homeobox gene, *Hox-2·1*. *Development* **99**, 603–617.

LAWRENCE, P. A. & MORATA, G. (1983). The elements of the Bithorax complex. *Cell* **35**, 595–601.

LE DOUARIN, N. (1982). *The Neural Crest*. Cambridge University Press.

LEVINE, M., HAFEN, E., GARBER, R. L. & GEHRING, W. J. (1983). Spatial distribution of *Antennapedia* transcripts during *Drosophila* development. *EMBO J.* **2**, 2037–2046.

LEWIS, E. B. (1978). A gene complex controlling segmentation in *Drosophila*. *Nature, Lond.* **276**, 565–570.

MCGINNIS, W., GARBER, R. L., WIRZ, J., KUROIWA, A. & GEHRING, W. J. (1984). A homologous protein-coding sequence in *Drosophila* homeotic genes and its conservation in other metazoons. *Cell* **37**, 403–408.

RUDDLE, F. H., HART, C. P. & MCGINNIS, W. (1985). Structural and functional aspects of the mammalian homeo-box sequences. *Trends in Genetics* **1**, 48–51.

RUGH, R. (1968). *The Mouse*. Minneapolis: Burgess.

SHARPE, P. T., MILLER, R., EVANS, E. P., BURTENSHAW, M. D. & GAUNT, S. J. (1988). Isolation and expression of a new mouse homeobox gene. *Development* **102**, 397–407.

STRUHL, G. (1984). A universal genetic key to body plan? *Nature, Lond.* **310**, 10–11.

STRUHL, G. & WHITE, R. A. H. (1985). Regulation of the *Ultrabithorax* gene of *Drosophila* by other bithorax complex genes. *Cell* **43**, 507.

TOTH, L. E., SLAWIN, K. L., PINTAR, J. E. & NGUYEN-HUU, M. C. (1987). Region-specific expression of mouse homeobox genes in the embryonic mesoderm and central nervous system. *Proc. natn. Acad. Sci. U.S.A.* **84**, 6790–6794.

UTSET, M. F., AWGULWITSCH, A., RUDDLE, F. H. & MCGINNIS, W. (1987). Region-specific expression of two mouse homeobox genes. *Science* **235**, 1379–1382.