Mouse Hox-3.4: homeobox sequence and embryonic expression patterns compared with other members of the Hox gene network

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Summary

A putative mouse homeobox gene (Hox-3.4) was previously identified 4 kb downstream of the Hox-3.3 (Hox-6.1)* gene (Sharpe et al. 1988). We have now sequenced the Hox-3.4 homeobox region. The predicted amino acid sequence shows highest degree of homology in the mouse with Hox-1.3 and Hox-2.1. This, together with similarities in the genomic organisation around these three genes, suggests that they are co-members of a subfamily, derived from a common ancestor. Hox-3.4 appears to be a homologue of the Xenopus Xlhbox5 and human cp11 genes (Fritz and De Robertis, 1988; Simeone et al. 1988). Using a panel of mouse-hamster somatic cell hybrids we have mapped the Hox-3.4 gene to chromosome 15.

From the results of in situ hybridization experiments, we describe the distribution of Hox-3.4 transcripts within the 12½ day mouse embryo, and we compare this with the distributions of transcripts shown by seven other members of the Hox gene network. We note three consistencies that underlie the patterns of expression shown by Hox-3.4. First, the anterior limits of Hox-3.4 transcripts in the embryo are related to the position of the Hox-3.4 gene within the Hox-3 locus. Second, the anterior limits of Hox-3.4 expression within the central nervous system are similar to those shown by subfamily homologues Hox-2.1 and Hox-1.3, although the tissue-specific patterns of expression for these three genes show many differences. Third, the patterns of Hox-3.4 expression within the spinal cord and the testis are very similar to those shown by a neighbouring Hox-3 gene (Hox-3.3), but they are quite different from those shown by Hox-1 genes (Hox-1.2, -1.3 and -1.4).

Key words: mouse embryo, homeobox, in situ hybridisation.

Introduction

The homeobox was originally identified as a 180 bp nucleotide sequence found at the 3' ends of many genes involved in the body plan development of Drosophila (reviewed by Gehring, 1987). The 180 bp sequence codes for a 60 amino acid 'homeo-domain' at the C-terminus of the proteins. This domain forms a helix-turn-helix structure (Laughon and Scott, 1984) (Otting et al. 1988) that binds selectively to DNA (Muller et al. 1989) and thereby permits the proteins to serve their probable role as transcription factors (Winslow et al. 1989). At least 25 different homeobox-containing genes have been identified in the mouse genome (e.g. Duboule and Dollé, 1989; Graham et al. 1989). The homeobox sequences of these genes are usually compared in the extent of their homology with the homeobox sequence of the archetypal Antennapedia (Antp) gene (e.g. Hart et al. 1987). Both in Drosophila and in vertebrates, homeobox sequences with close amino acid homology to Antp appear to lie within genes which are involved in determination of the body plan during development. These are the Drosophila homeotic and segmentation genes, and the mouse Hox genes (reviewed by Gehring, 1987; Akam, 1987; Dressler and Gruss, 1988).

The mouse Antp-like Hox genes are found in clusters in four chromosomal locations: Hox-1 on chromosome 6 (Colberg-Poley et al. 1985; Bucan et al. 1986), Hox-2 on chromosome 11 (Hart et al. 1985), Hox-3 on chromosome 15 (Breier et al. 1988; Schughart et al. 1989) and Hox-5 on chromosome 2 (Featherstone et al. 1988). The linear order of the genes in each cluster appears to resemble the linear order of Drosophila homeotic genes.
in the Antp and Bithorax complexes on chromosome 3, both in conservation of sequence and in anteroposterior domains of expression in the embryo (Boncinelli et al. 1988; Duboule and Dole, 1989; Graham et al. 1989).

The genes within each Hox cluster can be aligned, based on sequence comparison and intergene spacing, such that it is apparent that each cluster represents a duplication of an original ancestral cluster (Hart et al. 1987; Graham et al. 1989). The aligned genes with similar sequence characteristics thus form a 'subfamily' (Hart et al. 1987; Duboule and Dole, 1989). When members of a subfamily are compared in their transcription patterns, they have usually been found to display similar anteroposterior limits of expression in the embryo (Gaunt et al. 1988, 1989), but to show stage- and tissue-dependent differences in their regulation (Gaunt et al. 1989).

In this paper, we investigate the mouse homeobox gene Hox-3.4. The position of Hox-3.4 in the Hox-3 cluster, and the sequence of its homeobox, both indicate that this gene is a member of the Hox-1.3/3.2 subfamily. By use of in situ hybridization, we show that Hox-3.4, -1.3, and -2.1 each show clear similarities in the limits of their transcript domains along the anteroposterior axis of the embryo. Between the genes, however, we also note some striking tissue-dependent differences in their patterns of expression. We present evidence that tissue specificity in gene expression may, at least in part, be a function of the locus in which the homeogene is located.

Materials and methods

Isolation and mapping of a Hox-3.4 genomic clone

A Charon 35 mouse genomic library was screened under high-stringency conditions with a Hox-3.3 specific probe. Resulting recombinant clones were mapped using multiple restriction and partial digest mapping. A putative homeobox (Hox-3.4), 4 kb downstream of Hox-3.3 was detected by low stringency probing of phage digest blots with a Hox-3.3 homeobox probe (Fig. 1). A 2 kb BamH1–HindIII fragment containing the region of the putative Hox-3.4 homeobox was subcloned into pGem4, mapped and a 300 bp EcoRI–XbaI fragment found to hybridize to the homeobox probe was identified and subcloned for sequencing and production of riboprobes for in situ analysis.

Sequencing

The 300 bp EcoRI–XbaI fragment was subcloned into M13, mp18 and mp19 vectors and sequenced in both directions using the dideoxy method and the Sequenase enzyme (Sanger et al. 1977). The EcoRI site was found to be within the homeobox and so to obtain sequence 5' of this, the EcoRI–BamHI fragment was subcloned into mp19 and sequenced from the EcoRI site.

In situ hybridization

In situ hybridization to mouse embryo sections was carried out as previously described using 35S-labelled anti-sense and sense (control) riboprobes (Gaunt et al. 1986). The Hox-3.4 probe was derived by subcloning the 300 bp EcoRI–XbaI fragment into pSP72. Identical patterns of labelling were also obtained (not shown) by using a longer Hox-3.4 riboprobe that included the 300 bp sequence described above, but which also included a further 800 bases of 3' sequence (EcoRI–HindIII fragment). The Hox-2.1 probe was synthesised from the 450 bp BamH1–HindIII cDNA fragment (Krumlauf et al. 1987) subcloned into pGEM-2. The Hox-1.3 probe was as previously described (Gaunt et al. 1988). Each of the three riboprobes used in in situ hybridisation gave its own characteristic pattern of labelling (as described in this paper), showing that there was no significant cross-reactivity under the experimental conditions employed. The Hox-3.1, -3.3 (-6.1), -1.2, -1.4 and -1.5 probes were all as previously described (Gaunt et al. 1988).

Chromosomal assignment

20 μg samples of karyotyped mouse/harster somatic cell hybrid DNAs were digested with HindIII, run on a 1% agarose gel and Southern blotted onto Gene Screen Plus. The blots were hybridised under high-stringency conditions with the 1.1 kb EcoRI–HindIII Hox-3.4 fragment (1 M NaCl, 1% SDS, 10% polyethylene glycol) at 65°C and washed in 0.1× SSC (0.3 M sodium chloride/0.03 M sodium citrate), 1% SDS at 65°C. Aliquots of mouse and hamster DNA were also included as controls.

Results

Hox-3.4 homeobox sequence

The nucleotide sequence of Hox-3.4 homeobox (Fig. 2) shows greatest homology with the human cpl1 homeobox at 96% (Simeone et al. 1988). Highest homology with other mouse homeoboxes is with Hox-1.3 (77%) and Hox-2.1 (76%) (Odenwald et al. 1987; Krumlauf et al. 1987). Amino acid sequence homology (Fig. 3) suggests that Hox-3.4 is a homologue of the Xenopus Xlhbbox5 and human cpl1 genes since the three homeoboxes differ in only one amino acid at position 60 where there is a conservative change from serine to threonine between Hox-3.4 and Xlhbbox 5 (Simeone et al. 1988; Fritz and De Robertis, 1988). The Hox-3.4 and cpl1 homeoboxes have identical amino acid sequences (Simeone et al. 1988). Furthermore, the homology extends outside the homeobox sequence and Hox-3.4, Xlhbbox 5 and cpl1 all have an inframe stop codon at identical positions, 8 amino acids downstream of the homeobox. The amino acid sequences of Hox-1.3 and -2.1 homeoboxes are 90% homologous with -3.4. Little homology can be observed immediately upstream or downstream of the homeobox, and Hox-1.3 and -2.1 have inframe termination signals further downstream than Hox-3.4, at 16 amino acid and 15 amino acids, respectively. The nucleotide sequences of Hox-3.4 and
**Chromosomal location of Hox-3.4**

Previous results (Sharpe et al. 1988) indicated that Hox-3.3 is located on mouse chromosome 14. However, recent work by Schughart et al. (1989) has shown that this assignment was incorrect. These authors mapped Hox-3.3 to chromosome 15 using a panel of mouse-hamster somatic cell hybrid DNAs. In light of this evidence, we have probed the same panel of hybrids with a Hox-3.4 probe and confirmed that Hox-3.4 is on chromosome 15 (Table 1).

**Expression patterns for Hox-3.4 and subfamily comembers**

Fig. 4B shows the distribution of Hox-3.4 transcripts detected by in situ hybridization to a parasagittal section of the 12-day mouse embryo. For comparison with subfamily comembers, nearby sections of the same embryo are also shown after hybridization to Hox-2.1 (Fig. 4C) and Hox-1.3 (Fig. 4D) probes. Hox-3.4 transcripts were present in the central nervous system caudal to a boundary in the posterior myelencephalon (Fig. 4B). Hox-3.4 transcripts were not detected in parts of the brain anterior to the hindbrain boundary. The position of the Hox-3.4 transcript boundary within the hindbrain was very similar, and perhaps identical, to the position of the corresponding boundary for Hox-2.1 (Fig. 4C), but was apparently located slightly anterior to the boundary for Hox-1.3 (Fig. 4D). In the parasagittal plane of section examined, the abundance of Hox-3.4 transcripts was maintained along the length of the spinal cord (Fig. 4B). This distribution is similar to that found for Hox-2.1 (Fig. 4C), but Hox-1.3 transcripts showed, in contrast, a marked anterior-to-posterior fall in abundance over the anterior region of the spinal cord (Fig. 4D). This fall in transcript abundance was at the level of prevertebra 1 (pvl) in dorsal parts of the spinal cord, but was posterior to pv7 in ventral parts. Transverse sections (Fig. 4C) showed that this pattern of Hox-1.3 labelling in the cervical spinal cord was a consequence of low levels of transcripts in central, lateral and dorsal parts of the mantle layer, but with abundant Hox-1.3 transcripts in ventral parts. An adjacent transverse section hybridized to the Hox-3.4 probe showed a pattern similar to that shown for Hox-3.4 in Fig. 4B.
Table 1. Segregation of Hox 3.4 in mouse hamster hybrids

<table>
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<th>Cell line</th>
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<tr>
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</tr>
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<td>mAE 19</td>
<td>1, X</td>
<td>-</td>
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<tr>
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<td>2, 4, 7, 9, 12, 13, 19, X</td>
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<td>All autosomes, X</td>
<td>+</td>
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<tr>
<td>R44</td>
<td>17, 18, unidentified chromosomes</td>
<td>-</td>
</tr>
<tr>
<td>4B3/Ax3</td>
<td>2, 7, 12, 13*, 15, 19</td>
<td>+</td>
</tr>
<tr>
<td>C17B</td>
<td>1, 2, 3, 4, 7, 9, 12, 15, 17*, 19, X*</td>
<td>-</td>
</tr>
<tr>
<td>C11</td>
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<td>+</td>
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<td>-</td>
</tr>
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<td>2ac2</td>
<td>1, 2, 3, 4, 7, 8, 9, 10, 12, 13, 15, 16, 17, 18, 19, X</td>
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</tr>
<tr>
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<td>Ecm4e</td>
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<tr>
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<tr>
<td>Mia06</td>
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</tr>
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</table>

* Rearranged chromosomes.
† Chromosomes present in 15% of cells.

Fig. 4. (B) Hox-3.4, (C) Hox-2.1 and (D) Hox-1.3 transcripts detected by in situ hybridization within complete parasagittal sections of the 12.5 day mouse embryo. (A) bright-field, (B–D) dark-field illumination. The figures show nearby sections cut from the same embryo, my, myelencephalon; pv6, prevertebra 6, oes, oesophagus; ln, lung; sc, spinal cord; int, intestine; lv, liver; h, heart; tr, trachea. Boxes, areas examined in Fig. 8 and 10. Bar, 1.0 mm.

probe showed, in contrast to the Hox-1.3 pattern, strong labelling in both central and ventral regions of the mantle layer (Fig. 5B). Our observation on parasagittal sections (Fig. 4B, D; plane of section indicated on Fig. 5A) that Hox-3.4 expression in the cervical spinal cord extends dorsal to that of Hox-1.3 is therefore a consequence of strong Hox-3.4 expression in central parts of the mantle layer (Fig. 5B).

In the prevertebral column, Hox-3.4 transcripts (Fig. 6B) increased in abundance over prevertebrae 6 to 8 (pv6–8), were most abundant over pv8–14, and then declined over pv15–17. Low levels of labelling were clearly seen in all prevertebrae posterior to pv17. No evidence for Hox-3.4 labelling above background was detected in pv1–4, but in a few sections low levels of transcripts were apparently present in ventral parts of pv5. The anterior boundary of Hox-3.4 transcripts within the prevertebral column (detected as the rise in transcript abundance over pv6–8) was clearly posterior in position to the corresponding boundary for Hox-1.3 (Fig. 6C; Gaunt et al. 1988). Thus, Hox-1.3 transcripts increased in abundance from pv3–7, were detected in greatest abundance over pv7–14, and then declined in more posterior parts. Hox-2.1 transcripts in the prevertebral column (Fig. 7) were detected only weakly in comparison to Hox-3.4 and Hox-1.3. The distribution of labelling resembled most closely that shown by Hox-1.3, but transcripts were found to extend slightly more anteriorly, into the posterior part of pv2.

Hox-3.4 transcripts were detected only weakly above
Fig. 5. (B) Hox-3.4 and (C) Hox-1.3 transcripts detected over adjacent transverse sections from the mid-cervical region of the 12½ day spinal cord. (A) Bright-field view of section B, (B, C) dark-field illumination. ep, ependymal layer; cm, vm, central and ventral regions of the mantle layer (grey matter); eg, cervical ganglion; arrows indicate the plane of parasagittal sections shown in Fig. 4. Bar, 0.1 mm.

The background in the 12½ day lung (Fig. 4B, 8B). This finding differs from the intense labelling of the lung given by Hox-2.1 and Hox-1.3 probes (Fig. 4C, D; for Hox-2.1 see also Krumlauf et al. 1987; for Hox-1.3 see also Dony and Gruss, 1987). For all three genes, transcripts were confined to the outer mesodermal components of the lung, and were not detected above background over the lining endothelium.

Hox-3.4 transcripts were weak or absent in the trachea, but were readily detected in the oesophagus, apparently within the lining epithelium (Fig. 8). Hox-2.1 transcripts were similarly weak or absent in the trachea (Fig. 4C). Hox-1.3 transcripts were, in contrast, abundant in this structure (Fig. 4D). Unlike findings for Hox-3.4, Hox-2.1 and Hox-1.3 transcripts were not detected in the oesophagus (Fig. 4C, D).

Fig. 6. (B) Hox-3.4 and (C) Hox-1.3 transcript domains within the 12½ day prevertebral column. (A) Bright-field, (B, C) dark-field illumination. The hybridizations were made to nearby parasagittal sections. pv1, pv3, pv6, pv14, prevertebrae 1, 3, 6 and 14. Bar, 0.2 mm.

Hox-3.4 transcripts were present in dorsal parts of the stomach wall (Fig. 9B). As viewed in several different planes of section, ventral parts of the stomach showed little or no labelling above background. In certain planes of section, Hox-2.1 and Hox-1.3 showed distributions of transcripts within the stomach that were similarly restricted to dorsal parts (not shown; for Hox-2.1, see Holland and Hogan, 1988a), but in other planes of section, Hox-2.1 and Hox-1.3 transcripts were also present in ventral parts. Although most of the Hox-3.4 labelling was located in outer mesodermal derivatives, the endodermally derived lining epithelium was clearly labelled in anterior parts of the stomach (Fig. 9B). We have not yet established whether or not this Hox-3.4-positive epithelium in the stomach is continuous with Hox-3.4-positive oesophageal epithelium (Fig. 8). In more posterior parts of the gut, Hox-3.4 transcripts were detected in the mesodermal components of some
of the mantle layer. In contrast, Hox-1.2 (Fig. 11B) and Hox-1.4 transcripts (Fig. 11C; see also Galliot et al. 1989) were weak or absent in central regions of the mantle layer, and showed an overall distribution similar or identical to that of Hox 1.3 (Fig. 5C). Similarly, in parasagittal sections through the spinal cord, the distri-

(Fig. 10B), but not all, loops of the intestine. Some loops of intestine were similarly labelled by the Hox-2.1 probe (not shown), but most regions were labelled only over peripheral autonomic ganglia (as described by Holland and Hogan, 1988a). Hox-1.3 transcripts were abundant within mesodermal components of the intestine (not shown; see Dony and Gruss, 1987).

Hox-3.4 transcripts were abundant within the mesonephric and metanephric kidneys (Fig. 9B). Similar results (not shown) were obtained for Hox-2.1 and Hox-1.3 (for Hox-2.1: Holland and Hogan, 1988a; for Hox-1.3: Dony and Gruss, 1987). Hox-3.4 transcripts were detected in mesodermal components of the testis (Fig. 9B), but not in the developing testis cords (Fig. 9A), which contain the primordial germ cells and Sertoli cells. No specific labelling of the testis was given by Hox-2.1 and Hox-1.3 probes (not shown).

Some aspects of the Hox-3.4 expression pattern are also shown by Hox-3.3

In Fig. 11, we provide evidence that the difference noted between Hox-3.4 and Hox-1.3 expression in the spinal cord (Fig. 4B,D; 5B,C) might, in fact, be indicative of a more general difference between the expression patterns of Hox-3 and Hox-1 genes. Thus, in transverse sections of the cervical spinal cord, Hox-3.3 transcripts (Fig. 11A), like Hox-3.4 (Fig. 5B), were found to be abundant in both central and ventral parts

Figs 8–10. Hox-3.4 transcripts within the 124 day oesophagus and lung (Fig. 8), stomach, testis, mesonephric and metanephric kidneys (Fig. 9) and the intestine (Fig. 10). (A) Bright-field, (B) dark-field illumination. The fields shown in Fig. 8 and 10 are outlined on the bright-field view of the whole embryo (Fig. 4A). p, pharynx; oes, oesophagus; ln, lung; tr, trachea; int, intestine; lv, liver; t, testis; am, pm., anterior and posterior parts of the mesonephric kidney; met, metanephric kidney; st, stomach; D, V, A, P, dorsal, ventral, anterior and posterior aspects of the stomach. Bars, 0.2 mm.
Fig. 11. (A) Hox-3.3, (B) -1.2 and (C) -1.4 transcripts detected in transverse sections of the 12½ day mid-cervical spinal cord. These hybridizations, together with those shown in Fig. 5, were made to adjacent or nearby sections from the same embryo. Below, (D) Hox-3.3, (E) -1.2, (F) -1.3 and (G) -1.4 transcripts were localized within adjacent or nearby parasagittal sections taken through the cervical and anterior thoracic spinal cord of a 12½ day embryo. pv7, prevertebra 7. Bars, 0.2 mm.

The Hox-3.4 homeobox gene was originally identified by hybridisation of genomic phage blots at low stringency with the mouse Hox-1.5 probe. In order to confirm the presence of a homeobox sequence, we subcloned the 2 kb HindIII-BamHI fragment that hybridised with Hox-1.5 into pGem4. The fragment was further mapped and a region containing the putative homeobox sequenced. Except for the presence of two unusual amino acids, namely asparagine at positions 37 and 41, the Hox-3.4 sequence is a typical Antp-like mouse homeobox sequence. An inframe termination codon is located 8 amino acids downstream of the homeobox. The nucleotide sequence of the homeobox shows highest homology with the human cpl1 gene at 96%, which lies approximately 5 kb downstream of c8, and the Xenopus XlHbox5 gene (85%). Comparison of the amino acid sequence of these three genes suggests they are homologues, since all have termination codons in the same place and only one amino acid difference, this being a conservative change serine in Hox-3.4 to threonine in XlHbox5 at position 60. Comparing amino acid sequence of Hox-3.4 with other mouse homeobox sequences shows it to be most similar to Hox-2.1/1.3 subfamily (76%; Krumlauf et al. 1987) and -1.3 (77%; Odenwald et al. 1987; Fibi et al. 1988). We therefore assign Hox-3.4 to the Hox-2.1/1.3 subfamily (Fig. 13) This assignment is consistent with the assignment of human cpl1 to the Hox-2.1/1.3 subfamily (Boncinelli et al. 1988). It is also consistent with our earlier assignment of Hox-3.3 to the Hox-2.2/1.2 subfamily (Gaunt et al. 1988), and with the conclusions of Schugart et al. (1989).

Outside the homeobox, Hox-2.1 and -1.3 contain...
other regions where the amino acid sequences are very similar, particularly at the 5' end of the coding sequence (Fibi et al. 1988). For Hox-3.4, the sequence immediately outside the homeobox has little similarity to Hox-2.1 and Hox-1.3. The termination signals for Hox-2.1 and Hox-1.3 are further downstream (45 bp and 48 bp, respectively) than for Hox-3.4. Sequencing of Hox-3.4 cDNA will reveal the full extent of homology between Hox-3.4, Hox-2.1 and Hox-1.3.

The anterior limits of Hox-3.4 expression relate to gene position within the Hox-3 locus

By use of in situ hybridization experiments, we found that Hox-3.4 transcripts, like those of other Antp-like mouse homeogenes (e.g. Holland and Hogan, 1988b), are located within spatially restricted domains along the anteroposterior axis of the developing embryo. In the central nervous system, the anterior boundary of transcripts was located in the posterior myelencephalon of the hindbrain. In the prevertebral column, the anterior boundary of the transcript domain was located over pv6–8. When considered together with in situ hybridization results already published for two other genes of the Hox-3 cluster (for Hox-3.1: Awgulewitsch et al. 1986; Holland and Hogan, 1988a; Gaunt et al. 1988; for Hox-6.1 (3.3): Sharpe et al. 1988; Gaunt et al. 1988) our findings indicate that the relative positions of these three genes on chromosomal DNA correspond with the relative positions of their transcript domains in the developing embryo (Fig. 13). This conclusion for genes within the mouse Hox-3 locus is similar to conclusions made earlier for genes within the mouse Hox-1 locus (Gaunt et al. 1988), the Hox-2 locus (Graham et al. 1989) and the Hox-5 locus (Duboule and Döllé, 1989).

Hox-3.4 expression compared with that of its subfamily homologues, Hox-2.1 and Hox-1.3

In this paper, we have compared the transcript patterns for Hox-3.4 with those of its subfamily co-members, Hox-2.1 and Hox-1.3. Genes within a subfamily are true homologues, present in the genome following duplication of the ancestral homeogene cluster (Duboule and Döllé, 1989; Graham et al. 1989; Akam, 1989). In an earlier comparison of the expression patterns of three homologous genes within the Hox-1.4 subfamily (Hox-1.4, -2.6 and -5.1), Gaunt et al. (1989) made two principal conclusions. First, all subfamily members showed similar domains of transcription along the anteroposterior axis of the central nervous system and prevertebral column, and, second, the relative abundance of Hox-1.4, -2.6 and -5.1 transcripts varied between different tissues. These two conclusions are applicable, at least in part, to the observations now made upon the homologous genes of the Hox-3.4 subfamily. Thus, Hox-3.4 and Hox-2.1 apparently share the same anterior boundary for their transcripts within the myelencephalon. In addition, Hox-3.4, -2.1 and -1.3 each showed greatest abundance of transcripts over posterior cervical and anterior thoracic prevertebrae. The anterior boundaries within prevertebrae were especially similar for Hox-1.3 and Hox-2.1.

Fig. 12. (B) Hox-3.3, (C) -3.1, (D) -1.2 and (E) -1.5 transcripts within the region of the 12i day embryonic testis. (A) Bright-field, (B–E) dark-field illumination. The hybridizations were made to nearby sections from the same embryo. t, testis; met, metanephric kidney; st, stomach. Bar, 0.2 mm.
In spite of similarities described above, the transcript domains for Hox-3.4, -2.1 and -1.3 within the central nervous system and prevertebral column were not identical. Thus, the Hox-1.3 transcript boundary within the myelencephalon was apparently located posterior to the Hox-3.4/-2.1 boundary. We found earlier (Gaunt et al. 1988) that this boundary for Hox-1.3 was indistinguishable from the boundaries shown by genes of the Hox-1.2/-3.3 subfamily. Hox-3.4 also showed a clear difference from Hox-1.3 and Hox-2.1 in the anterior limits of its transcripts within the prevertebral column. The difference observed (a distance of three prevertebrae) is greater than the differences that have previously been found between members of the Hox-1.2/-3.3 (Gaunt et al. 1988) and Hox-1.4/-2.6/-5.1 (Gaunt et al. 1989) subfamilies.

Although Hox-3.4, -2.1 and -1.3 display some similarities in their domains of transcription along the anteroposterior axis, striking differences between genes were noted in their expression patterns within thoracic and abdominal organs. In the lung, for example, Hox-3.4 transcripts were detected only weakly, while Hox-2.1 and Hox-1.3 transcripts were abundant. In the trachea, Hox-3.4 and Hox-2.1 transcripts were weak, while Hox-1.3 transcripts were abundant. In the oesophagus and testis, Hox-3.4 transcripts, but not Hox-2.1 or Hox-1.3 transcripts, were readily detected. In an attempt to explain the functional significance of homeogene duplication to form subfamilies, Gaunt et al. (1989) proposed that the different members of a subfamily might provide developmental instructions to different tissues located at similar positions along the anteroposterior axis of the embryo. Applying this hypothesis to the findings now described in this paper, we would suggest, for example, that Hox-1.3 transcripts, but not Hox-3.4, might provide positional cues important for the development of the trachea and lung. In contrast, the oesophagus, a structure located at a similar anteroposterior position, might be instructed in its development by Hox-3.4 transcripts, but not Hox-1.3.

Tissue specificity in expression of a Hox gene may be shared with other members of its locus

Some of the observations presented in this paper indicate that tissue specificity in expression of a homeogene, known to be variable between members of a subfamily (vertical arrow in Fig. 13), might be rather constant for members of a locus, or at least for subclusters of adjacent genes within a locus (horizontal arrow in Fig. 13). This conclusion has been made independently by Dollé and Duboule (unpublished) from their recent studies on genes of the Hox-5 locus.

Tissue specificity in expression within a locus might be determined, for example, by distantly linked cis-acting control elements. Consistent with this, expression in the 12½ day embryonic testis has now been observed for two genes from the Hox-3 locus (Hox-3.3 and Hox-3.4: this paper) and three of the genes so far examined from the Hox-5 locus (Hox-5.2: Dollé and
Duboule, 1989; Hox-5.1: Gaunt et al. 1989; Hox-5.4: Dollé and Duboule, unpublished). We have not, in contrast, observed expression in the embryonic testis for any of six genes examined from the Hox-1 and Hox-2 loci (Hox-1.2, -1.3, -1.5, -2.1: this paper; Hox-1.4, -2.6: Gaunt et al. 1989). In this paper, we have also described patterns of expression within the central spinal cord that are apparently common either to Hox-3 genes or to Hox-1 genes. The difference observed was primarily in nervous tissue located in central regions of the mantle layer: Hox-3.3 and -3.4 were seen to be expressed strongly, while Hox-1.2, -1.3 and -1.4 were expressed weakly or not at all. This finding suggests that different Hox gene clusters might provide positional cues within different nerve tracts of the developing spinal cord. It is an interesting possibility, therefore, that the appearance of new nerve tracts during evolution of the vertebrate central nervous system might have been facilitated by serial duplication of the single, ancestral Hox gene cluster (Akam, 1989; Gaunt et al. 1989).

Several observations are less consistent with a proposal that a homeogene's tissue specificity in expression is determined simply by its locus. For example, Hox-3.3 and -3.4 transcripts are abundant in the embryonic testis, but Hox-3.1 transcripts are weak or absent (this paper); Hox-3.3 transcripts are abundant in the 12.4 day lung (Sharpe et al. 1988; Gaunt et al. 1988), but Hox-3.4 transcripts are detected only weakly in this structure (this paper); Hox-2.1 transcripts are limited to only a few loops of the 12.4 day intestine (this paper), but Hox-2.6 expression is detected in most or all of the gut (Gaunt et al. 1989); Hox-2.6 transcripts are abundant in the 12.4 day prevertebral column (Gaunt et al. 1989), but Hox-2.1 transcripts are only weakly detected (this paper). If, as discussed above, tissue specificity in homeogene expression is a function of the locus, or part of a locus, then it is clear that other, unknown factors may superimpose to modulate expression of individual member genes.

We thank Frank Ruddle and colleagues for the generous gift of the mouse/hamster cell hybrid DNAs and for originally pointing out the incorrect assignment of Hox-6.1 to chromosome 14, Robb Krumlauf for the Hox-2.1 probe and Denis Duboule for sharing unpublished results. This work was funded in part by the Wellcome Trust and Medical Research Council of Great Britain.

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(Accepted 5 March 1990)