

Expression of homeobox gene *Hox 1.1* during mouse embryogenesis

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Summary

Many of the genes controlling segmentation and pattern formation in *Drosophila* contain a conserved 183 bp sequence known as the homeobox. Homeobox sequences have been found in a range of metazoan species, including the vertebrates mouse and man. This striking conservation suggests that homeobox genes may play a fundamental role in developmental processes. If this is the case then it might be expected that vertebrate homeobox genes will be differentially expressed during embryogenesis and that the timing of their expression will coincide with major morphogenetic events. Here the spatial and temporal patterns of expression of murine homeobox genes will be explored, concentrating on the *Hox 1.1* gene as an example. Using *in situ* hybridization to localize RNA transcripts, it has been found that *Hox 1.1* is ex-

pressed in a region-specific manner during the formation and differentiation of the embryonic anteroposterior axis. Although striking patterns of expression of *Hox 1.1* and other homeobox genes are seen in overtly segmented structures of the embryo (i.e. somites, prevertebral elements, neural tube and dorsal spinal ganglia) expression is also seen in tissues with no obvious segmental origin. The results suggest that homeobox genes probably do not play an exclusive role in segmentation in vertebrates, but are consistent with a role in the assignment of positional identity along the axis of the embryo.

Key words: homeobox, embryogenesis, gene expression, pattern formation, segmentation.

Introduction

Genetic and molecular analyses have revealed that the development of the embryonic body plan in *Drosophila* relies on the proper expression and interaction of a number of genetic loci (reviewed in Gehring & Hiroshi, 1986; Gehring, 1987; Akam, 1987; and Scott & Carroll, 1987). These genes are involved in specifying the body axes and metamer organization of the embryo, including segment polarity (segmentation genes) and positional identity (homeotic genes). The important role played by these genes has been determined largely through a detailed examination of the phenotypes of mutant alleles, which in many cases produce quite dramatic alterations in the patterning of the embryonic body.

The molecular dissection of several genes from the *Antennapedia* and *Bithorax* gene complexes uncovered the existence in each gene of a conserved

183 bp sequence, the homeobox. The homeobox encodes a putative DNA-binding domain of the helix-turn-helix type (McGinnis *et al.* 1984a,b; Scott & Weiner, 1984), a notion that is supported by the *in vitro* DNA-binding properties of several homeobox gene products (Desplan *et al.* 1985), and their nuclear localization as established by immunostaining with specific antibodies against homeodomain peptides (White & Wilcox, 1984; Beachy *et al.* 1985; Carroll & Scott, 1985; Di Nardo *et al.* 1985). These observations strongly support the view that proteins containing the homeodomain exert their effects by directly regulating gene expression. The conservation of the homeobox motif has facilitated the isolation of additional developmentally important genes from *Drosophila*. In fact, there appear to be several classes of homeobox genes that can be distinguished by variant homeodomain sequences (Gehring, 1987; Scott & Carroll, 1987; Rushlow *et al.* 1987).

The homeobox conservation extends throughout the animal kingdom. It has been found, for example, in the genomes of echinoderms, annelids and chordates (McGinnis *et al.* 1984b; Holland & Hogan, 1986). Although to date very little is known about the function of homeobox genes in species other than *Drosophila*, their presence in such diverse and evolutionarily distant phyla argues that they play some conserved role in development.

At least 18 genes, the so-called *Hox* genes (Martin *et al.* 1987), have been isolated from the mouse that contain sequences related to the *Antennapedia*-type homeobox. Most, and perhaps all, of these genes are located in clusters on at least four different chromosomes (reviewed in Fienberg *et al.* 1987; Colberg-Poley *et al.* 1988; Holland & Hogan, 1988b). There appear to be additional genes, such as *En-1* and *En-2*, that have homeoboxes similar in sequence to divergent classes of *Drosophila* homeobox genes (in this case, the *Drosophila engrailed* gene) (Joyner & Martin, 1987). In addition, several other genes have been isolated by virtue of homology to conserved structural motifs present in other genes controlling morphogenetic events in *Drosophila*, such as the 'finger domains' from the *Krüppel* gene (Chowdhury *et al.* 1987) and the 'paired box' found in the *paired-gooseberry* genes (Deutsch *et al.* 1988). The discovery of more widespread conservation among several classes of developmental genes strongly suggests that such homologies are not simply fortuitous and raises the intriguing possibility that there may be classes of vertebrate regulatory genes, analogous to those in *Drosophila*, which are essential for vertebrate development.

The task of defining the function of the homeobox genes in vertebrates is a very challenging one, as presently there are no known existing mutations at any of these loci. Thus, current analyses focus on determining the patterns of gene expression in the hope that a possible function can be deduced. A role in the specification of positional information, suggested by analogy to *Drosophila* homeobox genes, implies that at least two predictions might be true. The *Drosophila* genes are restricted in their expression to specific regions of the embryo, generally corresponding to the regions or segments defective in mutants of these genes (Akam, 1987; Scott & Carroll, 1987). If the homeobox genes of vertebrates play some role in defining the embryonic body plan, then they might also display regional specificity in their expression during embryogenesis. This prediction has recently been verified as several murine homeobox genes have been found to be differentially expressed in the central nervous system of midgestation embryos in distinct, yet overlapping, spatial domains (reviewed by Holland & Hogan, 1988b).

The second prediction is that expression should coincide with the time when determinative events in pattern formation take place. In *Drosophila*, these decisions occur very early. In fact, considerable positional information is present in the egg at the time of fertilization, and most of the zygotically active segmentation and homeotic genes are expressed before, or shortly after, cellularization (reviewed in Akam, 1987; Scott & Carroll, 1987).

In the mouse, a highly regulative organism, determinative decisions appear to be made relatively late. For example, there is no obvious morphological polarity in the fertilized egg and individual blastomeres remain totipotent or pluripotent through initial cleavage events (reviewed in Rossant & Pederson, 1986). It is through the process of gastrulation, which begins in the mouse around 6.5 days *post coitum* (*p.c.*), that the anteroposterior axis is definitively established (reviewed in Hogan *et al.* 1985; Hogan *et al.* 1986).

Prior to gastrulation, the primitive ectoderm, the region that gives rise to all embryonic tissue, exists as a cylindrical layer of cells with no recognizable anteroposterior polarity. In a region of the primitive ectoderm called the primitive streak, some cells delaminate and migrate between the primitive ectoderm and visceral endoderm. These cells migrate both anteriorly and laterally to form the mesoderm of the embryo proper, and posteriorly, to give rise to the extraembryonic mesoderm lining the exocoelom and a structure known as the allantois. The allantois ultimately fuses with the chorion to form the placenta. This entire process initiates inductive tissue interactions leading to the derivation of neural tissue and organogenesis. One of the most striking features, however, involves the development of the axial structures of the embryo, particularly the sequential partitioning of paraxial mesoderm into the somites. Apart from dividing the mesoderm into metameric units, giving the embryo a segmented appearance, the somites appear to impose segmentation on the nervous system (Hogan *et al.* 1985). Once formed, the somites differentiate into three parts: the dermatome, the myotome and the sclerotome, which eventually form the dermis of the skin, the muscles of the trunk and the axial skeleton, respectively. Both the initial deposition and subsequent differentiation of the somites occur in a distinct rostrocaudal sequence which takes place over a period of several days (Rugh, 1968; Theiler, 1972).

Experimental embryology of both the mouse and the chick, which have very similar developmental strategies, indicates that the first evidence for restriction in developmental potential along the anteroposterior axis occurs at gastrulation (Kieny *et al.* 1972; Snow, 1981, 1985; Beddington, 1982; Bellairs *et al.*

1986). It is therefore important to determine the pattern of expression of the homeobox genes during this period of immense morphological change when the basic body plan of the embryo is established.

This article will describe the spatial pattern of expression of the *Hox 1.1* gene during mouse embryogenesis. Although the data will be described in more detail elsewhere (Mahon *et al.* 1989), the general conclusions presented here show that *Hox 1.1* is expressed in a region-specific manner around the time the axis of the embryo is formed. Taken together with the expression patterns of other *Hox* genes, a strong case can be made for a role for homeobox-containing genes in determining positional identity.

Spatial patterns of expression of *Hox 1.1*

The *Hox 1.1* gene is one of at least seven genes located in the *Hox 1* cluster on chromosome 6. It contains a single intron of 1.1 kb and produces a 2.4 kb mRNA transcript. The deduced protein sequence encoded by this gene is 229 amino acids long (Kessel *et al.* 1987). Immunostaining with antibodies made against *Hox 1.1* peptide indicates that the *Hox 1.1* gene product is a nuclear protein (Kessel *et al.* 1987), consistent with the nuclear localization of many *Drosophila* homeobox gene products and the putative DNA-binding properties suggested by protein sequence.

Northern blot analysis of RNA from embryos has indicated that the peak of *Hox 1.1* expression occurs at 12.5 days *p.c.* (Colberg-Poley *et al.* 1985). Spatial patterns of expression were analysed by *in situ* hybridization of ³⁵S-labelled *Hox 1.1* probes to tissue sections of mouse embryos. The results indicated that *Hox 1.1* is differentially expressed along the anteroposterior axis in 12.5-day mouse embryos, primarily in regions of the developing central and peripheral nervous system and in the axial skeleton.

Hybridization was detectable over the neural tube and adjacent dorsal spinal ganglia extending from the fourth cervical ganglion (C4) to the lower lumbar region (Fig. 1). The anterior limit of hybridization appeared quite distinct. However, the intensity of hybridization was variable in both the neural tube and ganglia along the longitudinal axis, appearing strongest in the anterior regions and gradually declining in more posterior regions. Hybridizing regions of the neuroectoderm derivatives were largely in alignment, although the neural tube slightly anterior to C4 showed some expression (note arrow in Fig. 1A).

Transcripts also accumulate in condensations of sclerotome cells that form the primordia of the vertebrae and ribs. As is the case in the neuroecto-

derm derivatives, the prevertebrae and associated rib primordia that express *Hox 1.1* are restricted along the anteroposterior axis to a region extending from the third thoracic element to the first lumbar element (i.e. 10–20th prevertebrae inclusive). It is intriguing that the regions expressing *Hox 1.1* in the neuroectoderm and in the mesoderm-derived tissues overlap but are not in complete register (diagrammed in Fig. 1A). Interestingly, sclerotome cells immediately surrounding the notochord do not express detectable levels of *Hox 1.1* transcripts (Fig. 1BII). In addition to the axial structures, *Hox 1.1* is also expressed in the mesoderm of the stomach at this stage and in the metanephric kidney at later stages.

The expression pattern within both the neural tube and axial skeleton changes as these tissues differentiate. Transcripts in the neural tube, uniformly distributed along the dorsal–ventral axis at 12.5 days *p.c.* (Fig. 1), become more prevalent along the dorsal region in 13.5-day-old embryos (Fig. 2A,B), while expression in the primordia of the skeletal elements diminishes as chondrification centres appear (not shown).

The striking regional specificity characterizing *Hox 1.1* expression in these relatively late stages of development is apparent in earlier stages as well. In 9.5-day embryos, *Hox 1.1* transcripts are prominent within the neuroectoderm and mesoderm derivatives in the posterior region of the embryo. Expression was detectable in the neural tube and neural crest (from which the spinal ganglia and rest of peripheral nervous system is derived) as well as in paraxial and lateral plate mesoderm. Expression was also seen in the mesenchyme of the anterior limb buds. Hybridization was initially quite strong in the buds at the time when they first appear, but became more diffuse by day 12.5 *p.c.* It is worth noting that the anterior limb buds originate in a region along the axis spanning several somites (somites 8–12) (Theiler, 1972) – within the anteroposterior domain of *Hox 1.1* expression.

Expression of *Hox 1.1* first becomes apparent in the posterior ectoderm and mesoderm when the embryo has acquired between 8 and 12 somites (8.0–8.5 days *p.c.*). However, transcripts were detected at earlier stages (7.5 days) in extraembryonic mesoderm (see below). Thus far, transcripts have not been detected in earlier, preimplantation, embryos.

Tissues expressing *Hox 1.1* in midgestation embryos are summarized in Table 1. *Hox 1.1* is more broadly expressed in early embryos in both the neuroectoderm and in the mesoderm. Striking, and somewhat transient, expression patterns evolve which ultimately become more restricted as development proceeds. This is most convincingly evident in

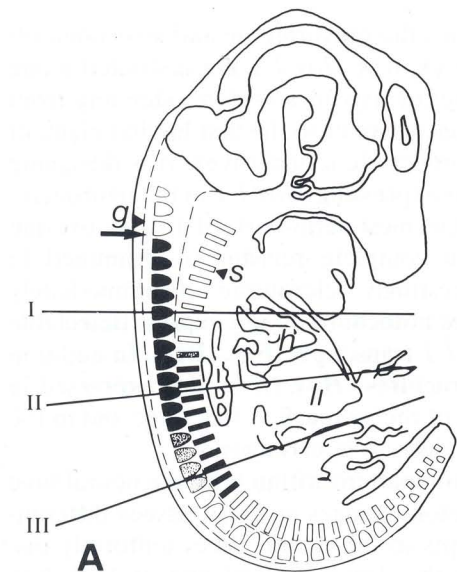


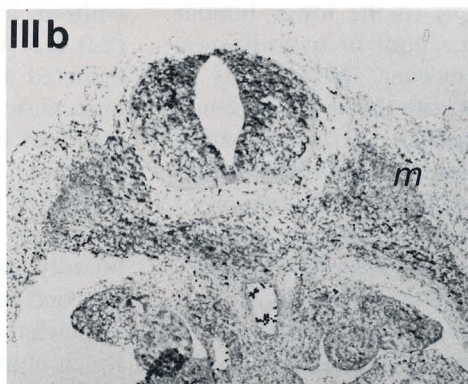
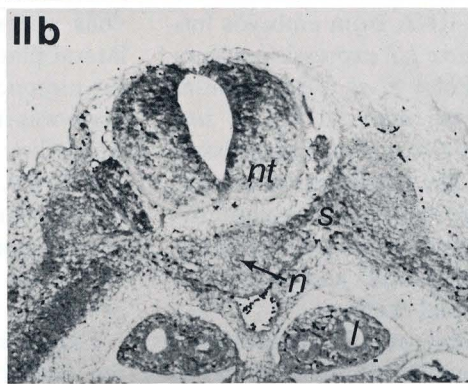
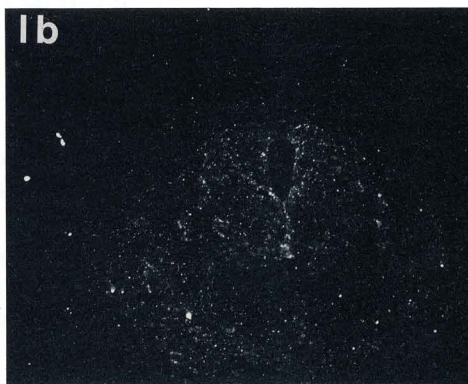
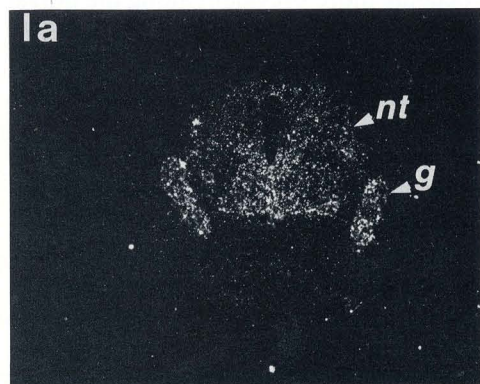
Fig. 1. (A) Schematic representation of the distribution of *Hox 1.1* transcripts in the dorsal spinal ganglia (*g*) and sclerotome-derived prevertebra (*s*) in the 12.5-day-old embryo as determined by an analysis of serial sections hybridized *in situ* with ^{35}S -labelled antisense *Hox 1.1* transcripts. Hybridizing regions are indicated by filled and stippled areas. Transcripts accumulate in the thoracic sclerotomes (T3–L1) and in the ganglia beginning at the fourth cervical ganglion (C4) and extending caudally. The rostral limit of hybridization over the neural tube is slightly anterior to C4 and is indicated by the arrow. The distribution of grains over the neural tube is similar to that of the adjacent ganglia, with the signal gradually decreasing in more posterior regions (as depicted by the stippled labelling). Lines I, II and III indicate planes of sectioning shown in B.

(B) *In situ* hybridization of representative cross-sections along the planes shown diagrammatically in A. (I.a) Dark-field image of a section through the midcervical region hybridized to *Hox 1.1* antisense transcripts. Neural tube and spinal ganglia are labelled. (b) An adjacent section hybridized to *Hox 1.1* sense transcripts. No specific labelling is seen. (II) Hybridization of *Hox 1.1* anti-sense probe to sections taken at

the level of the lung. The neural tube, spinal ganglia and sclerotome are labelled. Note that sclerotome cells immediately investing the notochord are unlabelled (arrow). (a) Dark-field illumination. (b) Bright-field illumination.

(III) Cross-section through the lower thoracic region hybridized to *Hox 1.1* anti-sense probe. In this region, the sclerotomes, including rib primordia, are very strongly labelled, whereas there is much less labelling of the neural tube and ganglia relative to more anterior sections. Note that the spinal nerve is unlabelled (arrow).

(a) Dark-field illumination. (b) Bright-field illumination. Exposure time: 10 days. *s*, sclerotome; *g*, ganglion; *nt*, neural tube; *m*, myotome; *n*, notochord; *l*, lung; *li*, liver; *h*, heart. Mag. 36 \times .



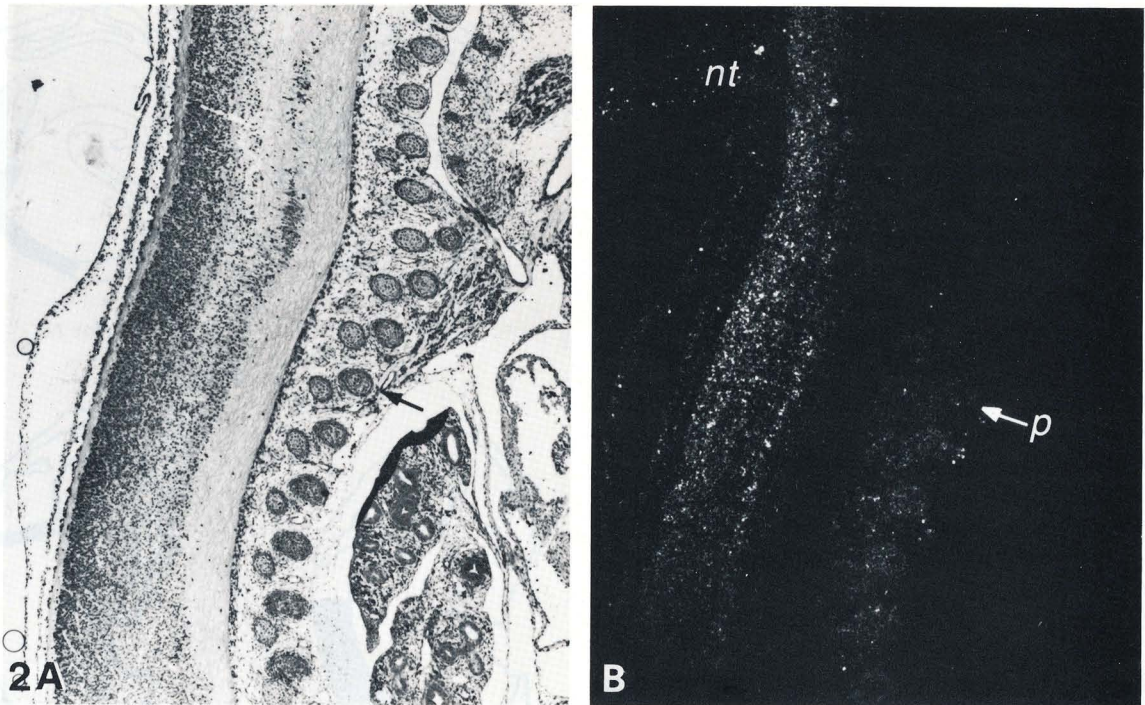


Fig. 2. Expression of *Hox 1.1* in the 13.5-day-old embryo, as demonstrated by *in situ* hybridization. Midsagittal section of a 13.5-day *p.c.* embryo hybridized with *Hox 1.1* probe and photographed under bright-field (A) and dark-field optics (B). The spatial limits of hybridization along the longitudinal axis are similar to the 12.5 day *p.c.* embryo. The arrow marks the most anterior prevertebral element to show hybridization. Transcripts become restricted to the more dorsal region of the neural tube at this stage. *nt*, neural tube; *p*, prevertebra. Mag. 50 \times .

Table 1. Embryonic expression of *Hox 1.1*

8–8.5 days <i>p.c.</i>	9.5 days <i>p.c.</i>	12.5 days <i>p.c.</i>
mesoderm	paraxial lateral plate	sclerotome limb bud stomach (metanephros*)
ectoderm	neural tube neural crest	neural tube dorsal signal ganglia

Embryonic germ layers and derivative tissues that express *Hox 1.1* at several embryonic stages. Arrows indicate developmental pathways.

* expressed later than day 12.5.

the mesoderm and its derivatives. In 9.5-day-old embryos, transcripts are seen in both paraxial and lateral plate mesoderm. As the somite differentiates, *Hox 1.1* expression is progressively turned off in the myotome and dermatome. Expression in the sclerotome persists until chondrification occurs. Likewise, the initially broad pattern of expression in the lateral plate mesoderm becomes restricted to specific derivatives, including the mesoderm of the stomach and metanephric kidney. It is important to note that these modulations in expression occur within a specific anteroposterior domain and do not appear to simply

reflect the differentiation state of the axial structures. If this were the case, expression might be expected to commence, for example, in the most anterior somites and subsequently proceed like a propagated wave along the anteroposterior axis over time, in parallel with the marked rostrocaudal sequence of somite development.

Anteroposterior domains of homeobox gene expression

Hox 1.1 has a spatial and temporal pattern of ex-

pression distinct from that of other homeobox genes. Transcripts from several genes have been localized by *in situ* hybridization to midgestation (12.5–13.5 day *p.c.*) embryos (Awgulewitsch *et al.* 1986; Gaunt *et al.* 1986; Dony & Gruss, 1987; Gaunt, 1987, 1988; Utset, 1987; Fainsod *et al.* 1987; Krumlauf *et al.* 1987; Toth *et al.* 1987; Holland & Hogan, 1988a; Sharpe *et al.* 1988; Breier *et al.* 1988; reviewed by Holland & Hogan, 1988b). Each gene analysed so far has been expressed in a spatially restricted manner in the developing central nervous system (CNS) and, in some cases, in a subset of the prevertebrae and visceral organs. It appears that expression in the viscera is also restricted according to position of origin along the longitudinal axis, in a manner consistent with the anteroposterior domain of expression particular to each gene (Dony & Gruss, 1987; Holland & Hogan, 1988a,b; Gaunt, 1988).

Comparison of the axial limits of expression of the various homeobox genes logically focuses on the CNS, since transcription is invariably detected there, and since many homeobox genes in *Drosophila* also show prominent CNS expression (Scott & Carroll, 1987). As is the case with *Hox 1.1*, the anterior boundaries of hybridization in the CNS are typically quite sharp and distinct for each gene, whereas the posterior limits are diffuse and hard to define (reviewed by Holland & Hogan, 1988b; Holland, 1988). Where expression is detected in the mesodermal derivatives such as the prevertebral elements, the rostrocaudal boundaries are not necessarily coincident with those in the nervous system. It remains a distinct possibility that the anteroposterior expression domains in the neuroectoderm and mesoderm are established (or maintained) independently. It is clear, however, that the homeobox genes constitute a class of genes, hitherto unknown, that are expressed in overlapping but noncoincident spatial domains along the embryonic axis.

Less certain is whether, in general, these distinct gene-specific expression domains are apparent at the time of initial axis formation, as the expression patterns of only a few genes (*Hox 1.1*, *1.5*, *2.1*, and *3.1*) have been determined at these early embryonic stages (Gaunt, 1987, 1988; Sharpe *et al.* 1988; Holland & Hogan, 1988a; Mahon *et al.* 1988). However, in the cases that have been analysed, it appears that the differences in regionalized expression apparent in later stages are evident during the time of axis formation in the late-gastrulating embryo (Gaunt, 1987, 1988; Holland & Hogan, 1988a; Mahon *et al.* 1988). Transcripts have not been detected prior to midgastrulation (7.5 days *p.c.*), but expression at levels below the limit of detection cannot be ruled out. The timing of the appearance of the transcripts in these cases correlates well with the anteroposterior

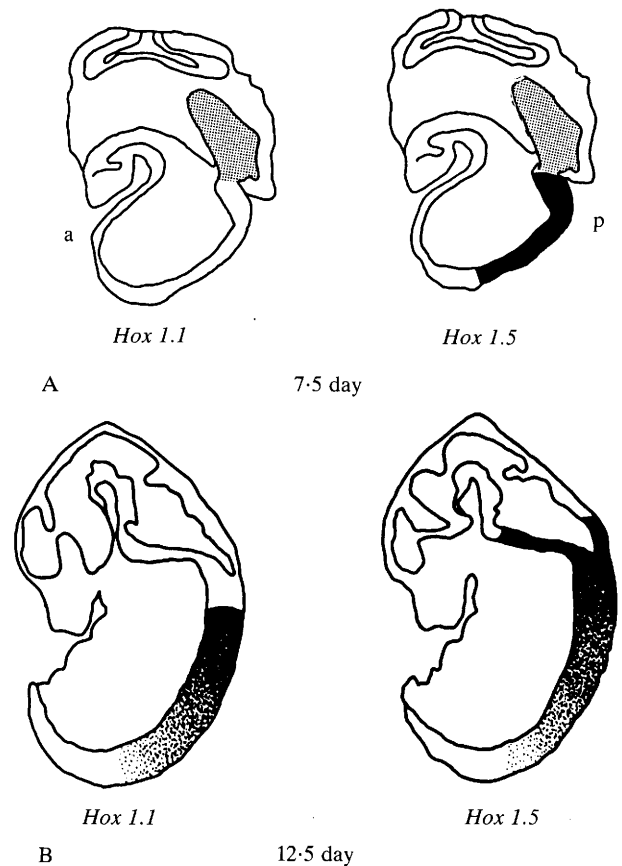


Fig. 3. Schematic representation of the domains of expression of homeobox genes *Hox 1.1* and *Hox 1.5* in two stages of embryogenesis. Shaded and stippled areas indicate the hybridizing regions and the relative intensity of hybridization observed. (A) In the 7.5-day *p.c.* embryo (approximately 2–3 somites), *Hox 1.1* transcripts are detectable in the allantois (stippled), whereas *Hox 1.5* transcripts are found in the posterior region of the embryo as well. *a*, posterior; *p*, posterior of embryo. (B) The anterior expression boundaries in the CNS of 12.5-day *p.c.* embryos extend from the fourth cervical ganglion (*Hox 1.1*) or myelencephalon (*Hox 1.5*) to more posterior regions of the neural tube. Embryos are depicted as sagittal sections. Hybridization domains were determined by hybridization of adjacent sections to either anti-sense *Hox 1.1* or *Hox 1.5* transcripts.

domains of expression seen in older embryos (Gaunt, 1988; Mahon *et al.* 1988).

As an example, shown in Fig. 3 is a schematic comparison of the expression pattern of *Hox 1.1* with that of the *Hox 1.5* gene. Localization of *Hox 1.5* transcripts has been conducted in two laboratories (Gaunt *et al.* 1986; Gaunt, 1987, 1988; Fainsod *et al.* 1988), and *Hox 1.5* probe was hybridized to parallel sections as a control in the *in situ* hybridization experiments with the *Hox 1.1* gene probe described here (Mahon *et al.* 1988). *Hox 1.5* has a more rostral limit of expression along the body axis than *Hox 1.1*.

In the CNS of the 12.5-day *p.c.* embryo, the boundary of expression of *Hox 1.5* is located in the myelencephalon and that of *Hox 1.1* is at the fourth cervical ganglion (Fig. 3B). In 7.5-day *p.c.* embryos, *Hox 1.5* is already expressed in the posterior region of the embryo, including the primitive streak, ectoderm and mesoderm, and to some extent in the allantois (Fig. 3A). In contrast, transcripts of *Hox 1.1* are detected in the allantois posterior to the embryo, but not in the embryonic region (Fig. 3D). Transcripts in the embryonic axis are found slightly later (day 8).

Studies of grafted and explanted embryonic tissue indicate that there is regionalization present in the embryos by midgastrulation as anterior and posterior regions of the primitive streak tend to give rise to anterior and posterior structures, respectively (Snow, 1981; Beddington, 1982; Tam & Beddington, 1987). It has been postulated that cells acquire positional information as they ingress through the primitive streak, supporting the view that the anteroposterior axis is established sequentially over the period of gastrulation (Hogan *et al.* 1985). As initially proposed by Gaunt (1987), if there is a rostrocaudal sequence to cellular determination along the body axis, genes with more anterior limits of hybridization, such as *Hox 1.5*, might be expressed at earlier times in development than those, like *Hox 1.1*, with more posterior boundaries. Transcripts from another posteriorly expressed gene, *Hox 3.1*, appear at approximately the same time and place as *Hox 1.1* (Gaunt, 1988).

Thus, regionally localized homeobox gene transcripts appear during the period when positional identities are thought to be established in the mouse embryo. Expression of homeobox genes has not been detected prior to midgastrulation. This argues that these genes are not involved in initiating major morphological events in axis formation, such as gastrulation and neural induction. Rather, these expression patterns are more consistent with a role in region-specific patterning along the embryonic axis. It will be essential for the spatial and temporal patterns of expression of other homeobox genes to be determined in order to ascertain if this early region-specificity is a general feature of homeobox gene expression.

Do homeobox genes play a role in segmentation?

The homeobox genes belong to a class of genes whose defining feature is region-specific expression. Although the *Hox* genes display cell-type-specific expression as well, this expression depends primarily upon position. Thus, two seemingly identical groups

of cells can differ significantly in the homeobox gene transcripts expressed depending upon their positions along the axis.

There are certain general features that the *Drosophila* and mouse homeobox genes share. Both are differentially expressed along the anteroposterior axis during development. At least some genes are expressed initially in broad patterns which eventually occupy more restricted domains in subsequent developmental stages. Different homeobox genes have distinct, and sometimes overlapping, regional domains of expression. This strongly suggests that there may be combinatorial interactions among mouse homeobox gene products similar to those proposed for *Drosophila*.

What is the nature of the role played by homeobox genes in vertebrate development? The mouse homeobox genes, including both *Hox* and *engrailed* (Davis *et al.* 1988) loci, and many *Drosophila* genes, are conspicuously expressed in the developing CNS, suggesting that they may play a conserved role in the development and/or diversification of cell types in the nervous system. However, since many of the *Drosophila* genes have a clear role in establishing the metameric body plan, the striking expression of murine homeobox genes in segmented structures of the embryo suggests that they may play a fundamental role in segmentation of the embryo. As has been discussed (Holland & Hogan, 1988a,b; Holland, 1988), an exclusive role in vertebrate segmentation seems unlikely. First, expression has been detected in several visceral tissues with no apparent segmental origin. Second, none of the *Hox* genes have been found to be expressed in all of the segmented structures along the axis, and consequently cannot be responsible for generating the segmental pattern. It seems more likely that homeobox gene products assign region-specific positional identity among the segmented structures along the rostrocaudal axis.

The hypothesis that homeobox genes specify positional identity along the anteroposterior axis seems consistent with the bulk of the available data. However, some expression patterns, particularly in adult tissues, are hard to reconcile with this hypothesis (Holland & Hogan, 1988a). Of course, in analysing RNA distribution, important questions concerning the distribution, longevity and state of modification of the protein products remain unanswered and merit further study. It seems likely, that the homeobox genes may play quite different roles in different cellular contexts and in combination with different sets of homeobox gene products. The observation that embryonic and neural activities of the *Drosophila* *ftz* gene can be distinguished (Doe *et al.* 1988) suggests that these functions may be very complex indeed.

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