Separate elements cause lineage restriction and specify boundaries of Hox-1.1 expression

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Summary

The Hox genes are a class of putative developmental control genes that are thought to be involved in the specification of positional identity along the anteroposterior axis of the vertebrate embryo. It is apparent from their expression pattern that their regulation is dependent upon positional information. In a previous analysis of the Hox-1.1 promoter in transgenic mice, we identified sequences that were sufficient to establish transgene expression in a specific region of the embryo. The construct used, however, did not contain enough regulatory sequences to reproduce all aspects of Hox-1.1 expression. In particular, neither a posterior boundary nor a restriction of expression to prevertebrae was achieved. Here we show correct regulation by Hox-1.1 sequences in transgenic mice and identify the elements responsible for different levels of control. Concomitant with the subdivision of mesodermal cells into different lineages during gastrulation and organogenesis, Hox-1.1 expression is restricted to successively smaller sets of cells. Distinct elements are required at different stages of development to execute this developmental programme. One position-responsive element (130 bp nontranslated leader) was shown to be crucial for the restriction of expression not only along the anteroposterior axis of the embryo, setting the posterior border, but also along the dorsventral axis of the neural tube and to the lineage giving rise to the prevertebrae. Thus, Hox-1.1 expression is established in a specific region of the embryo and in a specific lineage of the mesoderm by restricting the activity of the promoter by the combined effect of several regulatory elements.

Key words cell-lineage, gastrulation, homeobox, lacZ, pattern formation, transgenic mice.

Introduction

During the development of multicellular organisms, groups of cells acquire different fates and form distinct structures. How cells acquire the properties that allow them to follow the different developmental pathways is of fundamental importance to developmental biology.

A molecular analysis of developmental mutations in Drosophila melanogaster has led to the elucidation of the basic molecular mechanisms that establish the anteroposterior and the dorsventral axes of the larva (Akam, 1987; Ingham, 1988). A similar understanding of mammalian development, however, is still to be achieved. Several families of putative mammalian control genes have been isolated recently (Dressier and Gruss, 1988; Kessel and Gruss, 1990). Analyses of mouse mutants, ectopic expression in transgenic mice and experiments in Xenopus have shown that these genes serve a regulatory function during embryogenesis (Harvey and Melton, 1988; Wright et al. 1989; Balling et al. 1988, 1989; Kessel et al. 1990; Kessel and Gruss, 1990).

The Hox genes are likely candidates for factors assigning identities to different segments in mesoderm and neuroectoderm (Kessel and Gruss, 1990). It is apparent from their expression pattern that they are themselves regulated depending on the interpretation of positional information. A molecular analysis of the regulatory elements responsible for this position-specific activity will be a first step to unravel the process establishing the axes in the embryo.

During gastrulation, Hox-1.1 expression is established in a specific region of the neuroectoderm and the mesoderm. During organogenesis, expression in the mesoderm becomes progressively restricted to prevertebral cells and organs such as kidney and intestine (Mahon et al. 1988; Dressier and Gruss, 1989; Kessel, personal communication). At day 12 of development, Hox-1.1 is expressed in two overlapping domains in the neural tube, spinal ganglia (C5 to S4) and prevertebrae (T3 to T13).

A previous analysis in transgenic mice identified Hox-1.1 promoter sequences that were sufficient to establish transgene expression in a specific region of the embryo. The construct used, however, did not contain enough information to specify the posterior boundary and to restrict expression to prevertebrae, resulting in a uniform expression extending from the appropriate anterior limit of Hox-1.1 expression to the tail of the embryo. Here we show correct regulation by Hox-1.1
promoter sequences in transgenic mice and identify the elements that are necessary to specify the posterior boundary of expression and to restrict expression to prevertebrae.

Materials and methods

Recombinant DNA

To construct deletions in the promoter of 561acZ1, the sequences between the SaI site (position 0, see Puschel et al. 1990) and the following restriction sites were deleted (see also Fig. 1): BspMI at position 987 (561acZ1Bm), BamHI at 1255 (561acZ1B), SstI at 1449 (561acZ1Sst), XbaI at 1801 (561acZ1XII), Aul at 2152 (561acZ1A) and BamHI at 2047 (561acZ1B). To allow the construction of 561acZ4 (Fig. 3), oligonucleotides were synthesized containing the Hox-1.1 sequences (Kessel et al. 1987; Kessel and Gruss, 1988) from position 3639 (SacI site) to 3767 (ATG) and in addition several restriction sites from the pSP719-polylinker (sequence: GAGCTC... ATGAGTTCGGTACC). 561acZ4 was constructed by linking the following fragments in the indicated order: a 3.7 kbp SaI–SacI fragment from 561A (Puschel et al. 1990), the 130 bp oligonucleotide, a 1 kbp KpnI–ClaI fragment from pSPTlacZ-1 (A.W.P., unpublished), a 2 kbp ClaI–PstI fragment from pUR292 (Ruther and Muller-Hill, 1983), a 1.2 kbp PstI–BglII fragment from lam6 (Colberg-Poley et al. 1985; Kessel et al. 1987) containing a part of the first exon (from the PstI site to the splice site), the intron and the second exon up to the BglII site. The vector is based on 561acZ1. The cloning scheme results in the reconstitution of the genomic sequence from the SaI site up to the third codon of Hox-1.1 (Fig. 3A). The lacZ coding sequences were fused in frame N-terminally to the first three amino acids of Hox-1.1 and C-terminally to the Hox-1.1-coding sequences downstream of the PstI site in the first Hox-1.1 exon. All constructs contain the same 1.7 kbp fragment providing a polyadenylation signal as in 561acZ1 (Puschel et al. 1990). Several transcription-initiation sites of the Hox-1.1 gene are clustered around the SaI site (A.W.P., unpublished results). We assume that the same sites at the sites used in the transgenes. pSPTlacZ-1 was generated by cloning the BamHI–lacZ fragment of PMCl871 (Pharmacia) into the BamHI site of pSPT19 with the KpnI site of the polylinker upstream of the 5′ end of the lacZ reading frame. The plasmids 561acZ9 and 561acZ11 were generated by exchanging a ClaI–NsiI (ClaI site in lacZ, NsiI site in the second Hox-1.1 exon) or a SaI–ClaI fragment derived from 561acZ4 with the corresponding fragment of 561acZ1, respectively (Fig. 3A).

The fusion gene was separated from the vector for embryo injections by HindIII digestion. Restriction fragments were separated on agarose gels and isolated by electrophoresis. The fragment of interest was further purified by phenol:chloroform extraction, chloroform extraction, ethanol precipitation, gel filtration over a P-30 column (Biorad) and filtration through a 0.22 μm filter (Schleicher and Schuell).

Generation of transgenic mice

NMRI outbred mice and C57BL/6/DBA/F1, mice were purchased from the Zentralinstitut für Versuchstierzucht, Hannover (Germany). Transgenic mice were produced essentially as described by Hogan et al. (1986) 6-week-old NMRI female mice were superovulated by injecting intraperitoneally (i.p.) 5 i.u. of gonadotropin from pregnant mare serum 48 h prior to injecting 5 i.u. of human chorionic gonadotropin. Female mice were then mated with C57BL/6/DBA/F1 male mice. The next day, 1-cell embryos were flushed from the oviducts with M2 medium (Hogan et al. 1986) The eggs were freed of cumulus cells by hyaluronidase treatment. The male pronuclei of the fertilized eggs were microinjected with approximately 2 picoliters of DNA at a concentration of 2 ng/μl−1. The injected, fertilized eggs were then transferred to pseudopregnant recipient NMRI female mice. Transgenic mice were identified by Southern blot analysis of DNA extracted from mouse tail biopsies as described by Hogan et al. (1986). The expression pattern directed by the various constructs was analysed both in transient expression assays and in transgenic lines. For transient expression experiments, foster mice were killed 12 days after the retransfer and embryos (corresponding to the founder generation) were analysed for transgene activity. Placental DNA was used to assay for the presence of the transgene. Embryos were staged according to Theiler (1989).

X-Gal staining and histology

Embryos between day 7 and 12 (the day of detection of the vaginal plug was designated day zero) were fixed in 1% formaldehyde, 0.2% glutaraldehyde, 0.02% NaPO4, 1×PBS for 30 min at 4°C, followed by two washes in 1×PBS for 20 min at room temperature. Embryos were stained at 30°C in 1 μg/ml X-Gal, 5 mM K3Fe(CN)6, 5 mM K4Fe(CN)6, 2 mM MgCl2, 1×PBS overnight. For sectioning, stained embryos were fixed overnight in 4% formaldehyde in PBS, dehydrated and embedded in paraffin. Cryosections were air-dried, fixed at 4°C for 10 min like whole embryos and stained overnight as described above. Expression levels were estimated by the time required to obtain detectable levels of staining. This time varied from one hour to one day (data not shown). No correlation between copy number of the transgene and expression levels were observed.

Results

Analysis of the Hox-1.1 promoter

Previously we were able to reproduce the Hox-1.1 expression pattern partially in transgenic mice by using 3.6 kbp of promoter and 1.7 kbp 3′ sequences (561acZ1 in Puschel et al. 1990). A series of deletions of the 3.6 kbp promoter was analysed for expression in transgenic mice to delineate regulatory sequences further. Expression was analysed both in transient assays (the embryos were analysed for β-Gal activity at day 12 of gestation) as well as in lines (Fig. 1). Deletion of up to 1.94 kbp did not result in a change of the expression pattern or the frequency of transgenic mice expressing the transgene (Fig. 1). Deletion of another 110 bp (561acZ1B) changed both the pattern and the frequency considerably (Table 2, Figs 1, 2D,E,F). Three independent transgenic embryos (E254, E264, E372) obtained in transient assays did not show any boundary of expression either in ectoderm or in mesoderm (Fig. 1). A different result was observed when transgenic lines were analysed. The frequency of expressing lines was considerably reduced when compared to other constructs (Table 1). The 561acZ1B construct was expressed in only 3 out of 17 lines. Expression in one 561acZ1B line (L62) was similar to that of the 561acZ1 lines between days 8 and 10 (Fig. 2C). Expression at day 12 p.c. was considerably
reduced relative to earlier stages in terms of the extent of expressing tissues (data not shown). It is not clear if this is due to the construct or to the site of integration. The construct was particularly sensitive to influences of the integration site, as two of the three lines showed expression that was different from any pattern observed so far and was unique to the particular line (ectopic expression). A low number of expressing lines and high frequency of ectopic expression is commonly observed to the integration site, as two of the three lines showed expression that was different from any pattern observed so far and was unique to the particular line (ectopic expression). A low number of expressing lines and high frequency of ectopic expression is commonly observed.

Reproduction of Hox-1.1 regulation

In an attempt to reproduce fully the Hox-1.1 regulation, we created a construct that preserved the genomic configuration of the Hox-1.1 gene by retaining as much genomic sequence as possible. We replaced the coding sequence of the first Hox-1.1 exon by lacZ coding sequences (m6lacZ4). This resulted in the fusion of the first three codons of Hox-1.1 to the lacZ reading frame. lacZ was N-terminally fused in frame to the remainder of the Hox-1.1 reading frame. The resulting fusion protein contained the homeobox and is targeted to the nucleus in 3T3-cells (data not shown) and in the embryo (Fig. 6H), indicating the presence of a nuclear targeting signal in the homeobox (Hall et al. 1990), whereas lacZ-activity of the other constructs showed no particular subcellular localisation. In addition to the sequences of m6lacZ1, the m6lacZ4 construct contains 130bp 5'-nontranslated sequence, 96bp of the first Hox-1.1 exon, the Hox-1.1 intron and 146bp of the second exon all of which were deleted in m6lacZ1 (Fig. 3).

One embryo (E297) and three lines (L14, L30, L53) expressing the m6lacZ4 construct at moderate to high levels were recovered and analysed in detail Table 2, Figs 3B, 4B). This pattern was identical to that described for the endogenous Hox-1.1. The development of the embryonic expression pattern was analysed in the three transgenic lines (Table 2). The pattern in embryos of all three lines was identical on day 8 and 10. Line L14 did not show expression after day 10 p.c. Aside from some additional ectopic expression, the patterns of lines L30 and L53 were identical between day 10 and 12 p.c.

### Table 1. Expression in transgenic embryos (transient assays) at day 12 p.c.

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Fig. 2. *Hox-1.1* promoter analysis. Embryos were analysed for transgene expression by staining for β-Gal activity. Expression of the following constructs is shown: (A) m61acZ1S1: embryo E367. (B) m61acZ1A: embryo E386. (C) m61acZ1B: embryo from line L62 (day 10 p.c., expression in later stages was severely reduced). (D) m61acZ1B: embryo E254. (E) m61acZ1B: embryo E2564. (F) m61acZ1B: embryo E373.

Fig. 4. Identification of two regulatory elements in the *Hox-1.1* gene. Transgenic embryos were analysed for β-Gal activity at day 12 of development: (A) m61acZ1 (embryo from line L4, Piischel et al. 1990). (B) m61acZ4 (embryo E297). (C) m61acZ9 (embryo E325). (D) m61acZ11 (embryo from line L35).

Fig. 5. Lineage restriction by element C. Day 11 (A, B) and day 12 (C, D) embryos were stained for β-Gal activity. Anterior is to the left in A and C and to the right in B and D. White arrowheads delineate the boundaries of one mesodermal segment (sclerotome). (A) Expression in embryos of line L35 is seen throughout the sclerotome in the thoracic (A) and the caudal region (C). Expression directed by the m61acZ4 transgene is seen in a subset of sclerotomal cells in the thoracic (B, embryo of line L30) as well as in the caudal region (D, embryo of line L53). Also expression in the rib primordia is more limited in embryos expressing the m61acZ4 construct.
Mapping of the regulatory elements
The addition of two DNA segments to m6lacZ1 to create m6lacZ4 allowed the complete reproduction of Hox-1.1 regulation. We next determined if one or both of these two sequences are responsible for adding the necessary control elements to the m6lacZ1 construct. We constructed two plasmids where only one of these sequences was added, the 5' nontranslated leader (m6lacZ11: element B) or the intron (m6lacZ9: element C), respectively (Fig. 3B). The m6lacZ9 fragment was tested by transient expression experiments in transgenic embryos. Three transgenic embryos (E319, E324, E325) expressing the transgene were recovered (Table 2, Fig 4C). All three embryos had the same expression pattern, which was identical to that of m6lacZ1 (Fig. 3B).

One embryo (E347) and three lines (L17, L35 and L38) expressing the m6lacZ11 transgene were obtained (Table 2, Figs 3B, 4D). Line L17 showed ectopic expression starting at day 10 (not shown). With this exception, all integrations gave rise to the same expression pattern between days 8 and 12 p.c. The expression pattern was identical to that of m6lacZ4 with one exception. Whereas m6lacZ4 expression is restricted at day 11 to a subpopulation of sclerotomal cells (Figs 3B, 6G,H) and at day 12 to prevertebrae...
(Figs 5D, 6I), β-Gal activity directed by m6lacZ11 is seen in all sclerotomal cells within the expression domain at day 11 (Figs 5A,C, 6D,E) and throughout the prevertebral column, including the intervertebral disc anlagen at day 12 p.c. (Fig. 6F and not shown).

Change of the expression pattern during development

Expression of m6lacZ4 and m6lacZ11 was analysed in detail in transgenic lines (m6lacZ4: L30 and L53, m6lacZ11: L35 and L38) between days 8 and 12 p.c. (Fig. 7 and data not shown). The pattern directed by the two constructs was indistinguishable from day 8 to day 10. Expression is first detectable at day 8.5 in the posterior part of the neural plate (Fig. 7A). In contrast to m6lacZ1 no expression is detectable in the allantois. Subsequently transgene activity is detectable both in the presomitic and the intermediate mesoderm (day 9.0). At day 9.5, the transgenes exhibit the same characteristic for the construct, as the pattern (which was similar to L10) was different from the pattern seen in transent assays. Row % gives the percentage of transgenic lines expressing a particular construct (only characteristic pattern).

Table 2. Expression in embryos of transgenic lines

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Transgenic founder animals were identified by Southern analysis of tail-DNA. Transgenic males of the F0 generation (male founder) or the F1 generation (female founder) were mated to NMRI mice and expression in embryos was analysed between days 8 and 12 of gestation by staining for β-Gal activity. Row Σ gives the total number of transgenic founders obtained. Row Expr indicates the number of lines that expressed the transgene and # the designation of the line. The expression pattern was classified as characteristic for the construct (ch. +) when the same pattern was seen in several independent lines. Expression patterns that were unique to a single line were classified as ectopic (ec +) in some cases, the characteristic pattern was seen only from day 8 to 9.5 (the pattern of line L62 cannot be unequivocally classified as characteristic for the construct, as the pattern (which was similar to L10) was different from the pattern seen in transent assays. Row % gives the percentage of transgenic lines expressing a particular construct (only characteristic pattern).
between the patterns of m61acZ4 and m61acZ11. m61acZ11 activity is found throughout the sclerotome at day 11 and both in prevertebrae and intervertebral disc anlagen at day 12 (Fig. 6D–F). In contrast, m61acZ4 is active at day 11 in approximately the middle third of the sclerotome. At day 12, staining is seen only in prevertebrae in E297, L30 and L53 (Fig. 6G–I). Thus mesodermal expression of m61acZ4 is restricted to the lineage giving rise to prevertebrae (Fig. 8). Expression of both transgenes in the neural tube is detectable in the ventral half, whereas expression of m61acZ1 is expressed almost along the entire height of the tube (Fig. 9), making element B a potential target for inductive signals from the notochord and/or the floorplate which pattern the architecture of the neural tube (Placzek et al. 1990).


m61acZ4 expression at day 12

m61acZ4 expression in day 12.5 embryos was analysed in detail by serial sectioning of paraffin-embedded embryos. Expression is detectable in spinal ganglia and the adjacent neural tube from C5 to S4. In prevertebrae, a high level of expression is visible in prevertebrae T3 to T13. However, a low level of expression extends to the sacral region (Fig. 3B) and decreases gradually. Expression in T3 is very low, which is consistent with the in situ hybridization data obtained with Hox-1.1 probes (Puschel et al. 1990; Kessel, unpublished results). The expression can be seen in a small subpopulation of sclerotomal cells that contribute to the prevertebrae (Fig. 6G–I). The posterior boundary of transgene expression is more caudal than has been described for Hox-1.1. This may be due to the lower sensitivity of the in situ hybridisation method. To confirm the authenticity of transgene expression in the tail, a RNasea-mapping with a Hox-1.1 homeobox probe was performed with RNA isolated from tails and hindlimbs prepared from day 12 embryos. In both structures, Hox-1.1 RNA was clearly detectable, whereas RNA from heads did not contain Hox-1.1 message (data not shown). In addition the transgene is expressed in three other structures where Hox-1.1 expression was not reported so far and was probably missed by in situ hybridisation with RNA probes. Both the apical ectodermal ridge of the hindlimb and the ventral ridge of the tail express m61acZ4 (Figs 3B, 7D). In addition expression is seen in the dorsal aorta.

Discussion

Correct regulation does not require the context of the Hox-1 cluster

Using the m61acZ4 construct, we could reproduce fully the embryonic regulation of Hox-1.1 in transgenic mice. The context of the Hox-1 cluster was not necessary to reproduce qualitatively the expression pattern. However, we did not obtain correct regulation in terms of integration-site independence and copy-number dependence of expression levels. For this aspect of regulation, elements such as a locus control region (LCR) might be necessary as is the case for the /S-globin cluster and the Thy-1 gene (Behringer et al. 1990; Greaves et al. 1989; Grosveld et al. 1987; for a review see: Orkin, 1990). Competition of genes for interaction with the LCR in the /3-globin cluster is a prerequisite for correct regulation in the context of a gene cluster (Enver et al. 1990). The remarkable conservation of gene order between the four Hox clusters suggests that the context of the cluster might be important for this particular regulation of Hox gene expression (Gaunt and Singh, 1990). Furthermore, there is a clear correlation between the position of a gene within a Hox cluster and its anterior boundary of expression (Graham et al. 1989; Duboule and Dolle, 1989). The more 5′ a gene is located the more caudal is its anterior limit of expression in neural tube and prevertebrae. The spatial order of expression boundaries reflects the temporal order of activation in 3′-5′ direction during gastrulation. Thus, the gene order both in the /3-globin cluster and in the Hox clusters reflects the order of activation during development, which might indicate a common regulatory mechanism. Our observation that the temporal and spatial expression pattern can also be attained
outside of the cluster clearly indicates that individual Hox genes carry enough control elements in the vicinity of the gene to exert this control. Therefore, similar to the globin cluster control, a LCR may be required but would represent a different class of regulatory elements.

At least four elements are necessary for position-specific expression

Previously we have shown that 3.6kbp Hox-1.1 promoter sequences can direct expression of a marker gene to a specific region of an embryo with a discrete anterior boundary. Here we show that 1.7kbp promoter sequences are sufficient to specify the same expression pattern. Deletion of another 110bp (element A in lacZ1B, Fig. 10) results in a loss of the region-specific activity and a uniform expression throughout the embryo, indicating that this element is involved in specifying the anterior boundary of Hox-1.1 expression. Furthermore, this element is required for a correct pattern both in meso- and ectoderm. In one transgenic line (L62), the m6lacZ1B expression pattern was identical to that of m6lacZ1 indicating that element A is not the only regulatory element responsible for establishing the anterior boundary. L62 was the only m6lacZ1B line that showed this pattern. As the frequency of transgenic lines expressing the m6lacZ1B construct was significantly reduced compared to other constructs, we think that element A could function to ensure high levels of expression in addition to being involved in specifying the anterior border of expression. A result similar to the one described above was obtained when the m6lacZ1B was used to express lacZ transiently in transgenic zebrafish (Westerfield et al. in preparation). In general, the same elements that are required to restrict expression from the murine Hox-1.1 promoter in mice are also necessary in transgenic fish. In particular, deletion of element A resulted in uniform expression of lacZ activity in the fish embryo (Westerfield et al. in preparation).

Addition of the 5' nontranslated sequence (element B in Fig. 10) restricts expression driven by the promoter A+P in three aspects. Element B is necessary to set a posterior boundary both in mesoderm and ectoderm (anteroposterior restriction), to confine expression to sclerotomal cells (lineage restriction) and to restrict expression to the ventral half in the neural tube (dorsoventral restriction). At the moment, it cannot be decided if this element acts at the transcriptional level or by influencing mRNA stability. It also contains several conserved short open reading frames for which a function as a translational control element has been suggested (Bürglin et al. 1987; Kessel and Gruss, 1988).

A third element (element C in Fig. 10) is required to confine expression to prevertebrae. Element C contains a 100bp sequence which upon deletion results in a stabilisation of Hox-1.1 mRNA in F9 stem cells (Colberg-Poley et al. 1987; A.W.P., unpublished results). It is not clear if the same sequence is essential for activity of element C. The three elements A, B and C are required at different times during development to restrict expression of the Hox-1.1 promoter P (Figs 8, 10), reflecting different developmental decisions. We would expect that this mechanism of pattern formation is general for the Hox genes.

A preliminary analysis suggests a similar mechanism for regulation of the Hox-3.1 gene (Bieberch et al. 1990). The proposed mechanism, however, differs significantly from those postulated for the Hox-1.3 and HOX-5.1 genes (Zakany et al. 1988; Tuggle et al. 1990). These authors suggested that the additive effect of region-specific enhancers might be responsible for the specific expression patterns of these genes. This would

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**Fig. 10.** Three different regulatory elements restrict the activity of the Hox-1.1 promoter. The genomic structure of the Hox-1.1 gene and the location of the identified regulatory elements A, B, C and P are shown in the upper half. Element A (open box), a 110bp Aul (A)–BamHII (B) fragment; P, the 1590bp promoter extending from BamHII to SacI (Sc), Element B (stippled box), a 130bp fragment extending from the SacI site to the Hox-1.1 start codon (ATG). Element C (hatched box), a 1341bp PsI (Ps)–PvuII (Pv) fragment. The numbers indicate kbp of DNA. Transcribed sequences are shown as boxes, coding sequences as stippled boxes. Bg, BglII site; E, EcoRI site, Sc, SalI site; St, Stul site, X, Xbal site. The lower half shows a schematic representation of the patterns that are obtained with different transgenes. P, m6lacZ1B; PA, m6lacZ1; PAB, m6lacZ11, PABC, m6lacZ4. Stepwise addition of A, B and C restricts expression of the promoter P to ever smaller sets of cells.
be similar to the stripe-specific elements of several *Drosophila* segmentation genes (for a review see: Pankratz and Jackle, 1990). The transgenes used in these experiments, however, gave rise to only a very limited aspect of the endogenous expression pattern of the corresponding Hox genes. In particular, transgene activity was first seen considerably later than the onset of expression of the endogenous genes. The constructs employed may therefore not contain the major regulatory elements but sequences that are required after establishment of the pattern.

**Two elements restrict Hox-1.1 expression to the prevertebral cell lineage**

The axial structures of vertebrates are derived from mesodermal cells. After formation of somites from the presomitic mesoderm, cells at the ventromedial edge of the somites disperse and form the sclerotome, whereas cells from the dorsolateral part of the somites give rise to the dermamyotome. The sclerotomal cells will migrate towards the notochord and form prevertebrae and intervertebral discs. The precise cell lineage relationship between sclerotome and prevertebrae is still unknown. After formation of the sclerotome, a further differentiation into a rostral and caudal half can be observed (Verbout, 1985). This observation has been interpreted as a resegmentation process that results in a half-segment phase-shift during vertebral column formation (Remak, 1855). According to this view, the rostral half of one sclerotome and the caudal half of the preceding sclerotome fuse to form one vertebra, but the experimental evidence addressing this question is contradictory.

Expression of the m6 lacZ4 transgene is found in a subpopulation of cells in the central part of the sclerotome. It is possible that these are the progenitor cells for the prevertebrae and that the non-expressing cells will give rise to the intervertebral discs. The expression of m6 lacZ4 in the middle third of a mesodermal segment is consistent with a recent cell-lineage analysis of the sclerotomal cells. Sclerotomal cells derived from one somite contribute to only one hemivertebra and the adjacent halves of the intervertebral discs in chicken (C. Stern, personal communication). The expression pattern of *Hox-1.1* and m6 lacZ4 in the sclerotome and the cell-lineage study argue against the occurrence of resegmentation.

**Conclusions**

The identification of several position-responsive sequences now enables the identification of the trans-acting factors conferring positional information. So far the analysis of cis-acting elements has shown that several mechanisms act consecutively at different times during development to direct expression of a gene to a specific structure and a distinct region relative to the anteroposterior axis. Further dissection of the promoter should reveal additional elements involved in specifying the anterior boundary. In addition, it will be interesting to try to separate elements acting specifically in ectoderm or mesoderm.

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