Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 4760–4764, July 1988
Developmental Biology

Hox-5.1 defines a homeobox-containing gene locus on mouse chromosome 2

(*Hox-1.4*/human homolog/spinal cord/prevertebrae)

MARK S. FEATHERSTONE*, AGNÈS BARON*, STEPHEN J. GAUNT†, MARIE-GENEVIÈVE MATTEI‡, AND DENIS DUBOULE*§

*Laboratoire de Génétique Moléculaire des Eucaryotes du Centre National de la Recherche Scientifique, Unité 184 de l'Institut National de la Santé et de la Recherche Médicale, Faculté de Médecine, 11, rue Humann, 67085 Strasbourg Cédex, France; †Department of Molecular Embryology, Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT, United Kingdom; and ‡Unité 242 de Physiopathologie Chromosomique de l'Institut National de la Santé et de la Recherche Médicale, Hopital d'Enfants de la Timone, 13385 Marseille Cédex 5, France

Communicated by Pierre Chambon, February 22, 1988

ABSTRACT **We have isolated a murine homeobox-containing gene, *Hox-5.1*, by virtue of its relatedness to the *Hox-1.4* gene. *In situ* hybridization to metaphase spreads mapped *Hox-5.1* to band D of mouse chromosome 2. Sequence comparisons indicate that *Hox-5.1* is the murine homolog of the human *C13* homeobox-containing gene. *Hox-5.1* also bears significant similarity to the *Xenopus Xhox-1A* homeobox-containing gene and the *Drosophila* deformed homeotic gene at N-terminal and homeobox regions. Hox-5.1 transcripts were detected in mouse embryos, in adult mouse testis, kidney, heart, and intestine, and in mouse embryonal carcinoma cells treated with retinoic acid. *In situ* hybridization to sections from whole mouse embryos revealed *Hox-5.1* expression in spinal cord and prevertebrae.**

The genetics of pattern formation in the vertebrate embryo is still poorly understood. This is largely due to difficulties in obtaining developmental mutants and in embryo manipulation. One approach involves the identification of genes similar to those known to direct pattern formation in other organisms. Homeobox-containing genes (homeogenes) have a common conserved open reading frame, the homeobox (1, 2), that is also found in a number of fundamentally important developmental genes in insects (for review, see ref. 3). Homeogenes have thus attracted interest and have been isolated from mice, humans, *Xenopus*, and other vertebrates (4–14). Comparative (15, 16) and functional (17, 18) studies indicate that the translated homeobox domain has a DNA-binding structure and that homeogene products localize to the nucleus (19). They thus have expected characteristics of gene regulators. Homeogene transcripts are detected in a tissue- and region-specific manner in the vertebrate embryo, undergo qualitative and quantitative changes during embryogenesis, and are limited to specific tissue and cell types of the adult mouse. Their expression has also been evoked in a temporally regulated manner in embryonal carcinoma cells treated with chemical inducers of differentiation (9, 20–22).

Two homeogene complexes, *Hox-1* (4, 10, 23) and *Hox-2* (8), have been detected in the mouse genome on chromosomes 6 (4, 23, 24) and 11 (25), respectively. Other homeogenes have been described on chromosomes 12 (26) and 15 (13). Here we describe *Hox-5.1*, a member of a homeogene complex (*Hox-5*) that maps to mouse chromosome 2.¶

MATERIALS AND METHODS

A day-10 embryonic mouse cDNA library (27) was screened with a probe derived from *Hox-1.4* homeobox sequences (23). Positive clones were identified at a frequency of 1 in 150,000 clones. A 170-base-pair (bp) fragment (at the 3′ end of the first exon in Fig. 1) was used to rescreen the library resulting in positive clones at a frequency of 1 in 200,000 clones. A 500-bp fragment upstream of the *Hox-5.1* homeobox was used to screen the mouse genomic cosmid library pcos2 EMBL (ref. 28, a gift from H. Lehrach, Imperial Cancer Research Fund, London), and 3 positive clones were recovered from approximately two genome equivalents. Details of the screening, Southern blots, and sequencing have been reported (20, 23). Chromosome mapping was as described (29). The probe was ^{3}H-labeled by nick-translation (1.8×10^8 cpm/μg) of a plasmid consisting of fragment b (Fig. 1) subcloned in the pUC vector.

RNA isolation and S1 nuclease analysis were as described (20, 23). P19 cells (30) were a gift from M. W. McBurney (University of Ottawa, Ottawa). Poly(A)$^+$ RNA was prepared by oligo(dT)-cellulose chromatography. For RNA gel blots, 5 μg of poly(A)$^+$ RNA or 15 μg of poly(A)$^-$ RNA were electrophoresed in formaldehyde/agarose gels (31), transferred to Hybond-N sheets (Amersham), hybridized to probe b (Fig. 1), and stringently washed as described by the manufacturer. The same blots were rehybridized with a probe for a triose phosphate isomerase pseudogene (a gift from M. Methali, Laboratoire de Génétique Moléculaire des Eucaryotes, Strasbourg, France). *In situ* hybridizations were as described (32). For ^{35}S-labeled probes, the 3′ 500 bp of probe a (Fig. 1) was cloned into the vector pGem-1 (Promega Biotec, Madison, WI). Sense and anti-sense probes were obtained by transcription *in vitro* from the T7 and SP6 promoters, respectively.

RESULTS

A Homeogene on Mouse Chromosome 2. Sequence comparison of Hox-5.1 cDNA clones with relevant regions of a genomic 5.5-kilobase (kb) *Bam*HI fragment revealed two exons separated by an intron of ≈500 bp (Fig. 1). A conceptual translation generated a single long open reading frame and placed a *Hox-1.4*-like homeobox 28 nucleotides downstream from an acceptor splice site, an arrangement similar to other reported homeogenes. S1 nuclease and RNase protection analyses (data not shown) allowed the 5′ border of the first exon to be extended to the vicinity of an upstream *Eco*RI site (Fig. 1). Probes a and b (Fig. 1) were used in genomic Southern blots and gave rise to unique bands (data not shown). Probe b was, therefore, used for chromosome mapping by *in situ* hybridization (Fig. 2). Of 120

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

§To whom reprint requests should be addressed.

¶The sequence reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03770).

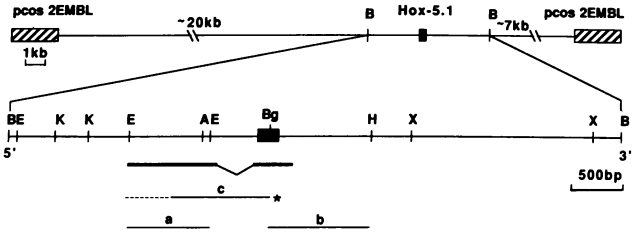

FIG. 1. *Hox-5.1* genomic organization. The upper line shows 33 kb of mouse genomic DNA inserted into a cosmid vector (hatched boxes). Position of a *Bam*HI fragment containing the *Hox-5.1* homeobox (solid box) is shown. The lower line represents the ≈5.5-kb *Bam*HI fragment carrying the *Hox-5.1* homeobox (solid box) and showing positions of *Bam*HI (B), *Eco*RI (E), *Kpn* I (K), *Acc* I (A), *Bgl* II (Bg), *Hin*dIII (H), and *Xba* I (X) restriction sites. The thick lines immediately below give the known boundaries of the two *Hox-5.1* exons. Transcription is from left to right. Fragments a and b represent genomic fragments subcloned and used as probes. Fragment c represents the subclone used as a probe for S1 analyses. It was end-labeled at the *Bgl* II site (asterisk) and included upstream sequences through the intron and first exon and then into the plasmid vector (dashed line).

metaphase spreads examined, there were 308 silver grains associated with chromosomes, and 53 of these (17.2%) were located on chromosome 2. Moreover, grain distribution was not random; 68% mapped to the C3→E1 region of chromosome 2, with a peak in the D band (Fig. 2*B*). These data strongly suggest that this gene lies within or near band D of mouse chromosome 2 and is thus designated as *Hox-5.1* in accordance with the accepted procedure (33).

A comparison of the 250 codons of *Hox-5.1* with other homeogenes revealed 90% DNA and 93% protein sequence identity with the human gene *C13* (34) (Fig. 3). *Hox-5.1* is thus the murine homolog of *C13*. This is further supported by a continuation of the DNA sequence similarity well into noncoding sequences. The amino acid sequence of Hox-5.1 also shows similarity to the products of the *Drosophila* homeotic gene deformed (36) and the *Xenopus* homeogene *Xhox-1A* (37) at N-terminal, homeobox domain, and interval regions. Moreover, the *Hox-5.1* homeobox is closely related to those of *Hox-1.4* (97%; ref. 23) and *Hox-2.6* (93%; R. Krumlauf, personal communication).

Expression of *Hox-5.1*. To examine *Hox-5.1* expression in development, embryonic RNA was prepared for RNA gel blot analysis. By using probe b (Fig. 1), Hox-5.1 transcripts were readily detected in poly(A)$^+$ RNA (Fig. 4). Multiple transcripts corresponding to 4.2 kb, ≈2.6 kb, and a less-abundant species at ≈1.4-kb, were observed with poly(A)$^+$ RNA at day 11. Although a Hox-5.1 species slightly larger than 4.2 kb may be present, this would be obscured by hybridization to low levels of 28S rRNA in the poly(A)$^+$ fraction. Signals from the two smaller transcripts are markedly decreased by days 13 and 15.

These results were extended by S1 nuclease analysis with the end-labeled probe c (Fig. 1). Poly(A)$^+$ embryonic RNA protected ≈170 nucleotides, which was most intense at day 11 and decreased by day 15 (Fig. 5). These results confirmed the presence of a splice-acceptor site located 170 nucleotides upstream of the *Bgl* II site in the *Hox-5.1* homeobox and also indicated that most of the Hox-5.1 transcripts are processed at this splice site. As for other homeogenes, the amount of Hox-5.1 transcript was increased in F9 and P19 embryonal carcinoma cells treated for 24 hr with the differentiation-inducing agent retinoic acid (data not shown). When poly(A)$^+$ RNA from eight adult tissues was used for S1 analysis, strong specific protection was observed for testis and kidney, whereas weak protection was observed for intestine and heart (Fig. 5). Thus, like other murine homeogenes, *Hox-5.1* displays a tissue specificity of expression in the adult. To localize *Hox-5.1* expression in the embryo, we used *in situ* hybridization. Hox-5.1 transcripts were detected in the prevertebrae in sagittal sections of day-12.5 embryos (Fig. 6). Silver grain density was especially high over the cervical prevertebrae, including that of the axis. Anterior to this point, however, the grain density repeatedly dropped, such that the prevertebra for the atlas was not labeled above background. This defines an anterior boundary for *Hox-5.1* expression between the prevertebrae for the developing axis and atlas. Hox-5.1 transcripts were also clearly present in the embryonic central nervous system. High silver grain density was observed over the spinal cord and over the most posterior part of the hindbrain (Fig. 6). Thus, *Hox-5.1* is expressed in discrete embryonic structures of mesodermal and ectodermal origin. Experiments with a sense (control) probe on similar sections showed no specific labeling (data not shown).

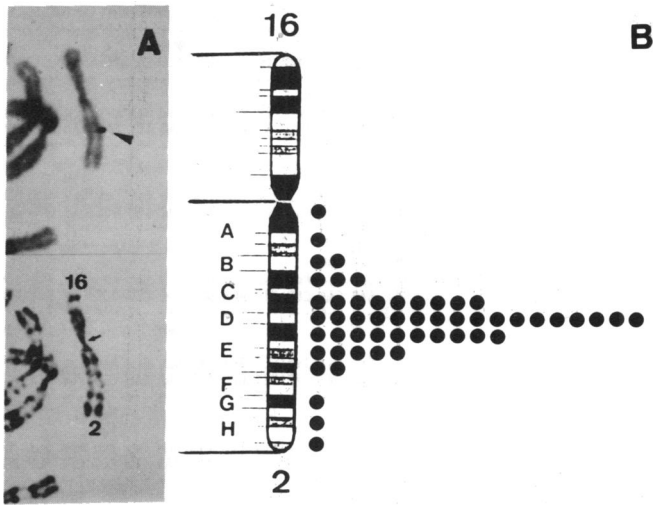

FIG. 2. (*A*) Localization of the *Hox-5* locus in the mouse genome by *in situ* hybridization in a representative metaphase spread showing the specific site of hybridization on chromosome 2. In the upper micrograph, the arrowhead indicates silver grains on Giemsa-stained chromosomes after autoradiography. In the lower micrograph, chromosomes with silver grains were subsequently identified by R-banding (fluorochrome/photolysis/Giemsa method). Arrow points to the region of centromeric fusion between chromosomes 2 and 16. (*B*) G-band diagram of WMP mouse Rb (2;16) chromosome, showing the distribution of silver grains on 53 labeled chromosomes. Most of the grains are localized to [C3–D–E1] region with a maximum in the D band.

DISCUSSION

This paper describes the murine homeogene *Hox-5.1*. *In situ* hybridization to metaphase chromosomes mapped this gene to mouse chromosome 2, which has not been reported to carry homeobox-containing genes. In addition to *Hox-5.1*, the two overlapping cosmids (defining a region of ≈52 kb) contain other homeobox sequences (unpublished results). Two murine genes highly similar to *Hox-5.1* lie within the *Hox-1* locus on chromosome 6 (*Hox-1.4*; B. Galliot, personal communication) and the *Hox-2* locus on chromosome 11 (*Hox-2.6*; R. Krumlauf, personal communication). Thus, *Hox-5.1*-like genes are represented in at least three murine homeogene complexes. It has been suggested that the *Hox-1* and *Hox-2* loci arose as a result of a large-scale duplication event on the basis of the conserved order and sequence of homeogene counterparts in the two clusters (38, 39). Our results indicate that duplication may have resulted in a third battery of homeogene homologs on mouse chromosome 2.

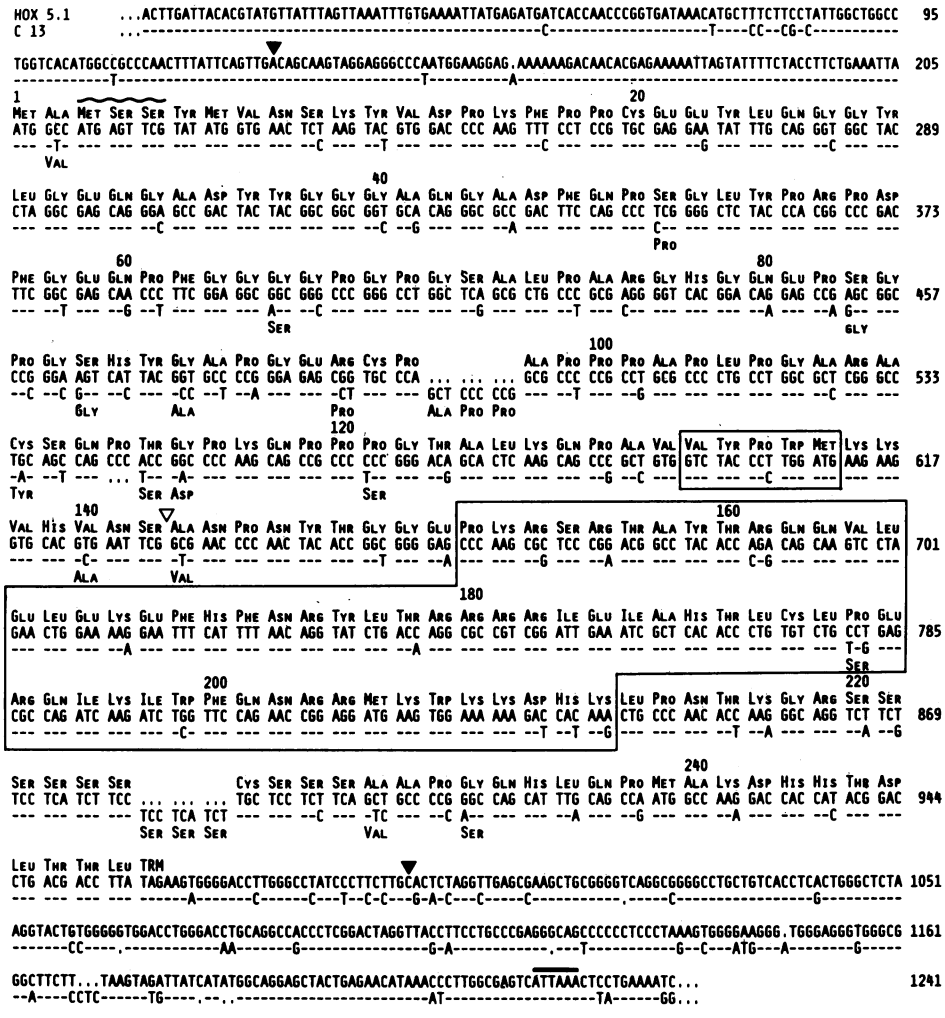

FIG. 3. Transcribed sequences of *Hox-5.1* and human gene *C13*. The nucleotide and amino acid sequences of *Hox-5.1* (top line) and *C13* (34) (bottom line) are presented. Differences are indicated as nucleotide and amino acid changes and deletions (dots). The beginning and end of *Hox-5.1* sequences initially obtained from cDNA clones are indicated by inverted solid triangles. Nucleotide numbers are given on the right, and amino acid numbers are placed over the sequence. The large and small boxed regions enclose the homeobox and a conserved pentapeptide-encoding sequence found on the 5′ side of the homeobox in both *Drosophila* and vertebrates (34). The position of the splice site is given by an open inverted triangle and is situated between the upstream box and the homeobox, as is typical for homeobox-containing genes. The wavy line marks the amino acid sequence Met-Ser-Ser conserved at the N terminus of homeobox-containing gene products. Note that the nucleotide sequence around this methionine codon is better related to the consensus sequence of Kozak (35) than that around another possible initiation codon at position 1. The *C13* (34) polyadenylylation site (ATTAAA), present in *Hox-5.1*, is overlined. TRM, termination codon.

Homeogenes at other positions in the mouse genome, for example the *Hox-3* complex (13, 21), may also be expected to define clusters with some similarity to the homologous *Hox-1* and *-2* complexes (39).

Hox-5.1 and its human homolog *C13* (34) share 93% identity at the amino acid level over the entire coding region, have an intron of similar size and position, and are highly related in 5′- and 3′-untranslated sequences. As in the mouse, the human *Hox-5.1* gene maps to chromosome 2 (2q31→q37) (40), although no clear cases of synteny have been reported between these two chromosomes.

Similar to *Hox-1.4*, to *Hox-2.6*, and to *Xhox-1A* (37), *Hox-5.1* shows regions of significant similarity with the *Drosophila* homeotic gene *Dfd*. Interestingly, the N-terminal region of similarity among *Dfd, Xhox-1A*, and *Hox-5.1* lies within a domain of unknown function that shows conservation among most of the murine homeogenes reported (39, 41).

Hox-5.1 is expressed in multiple RNA species as early as day 11 of gestation in a temporally regulated manner. We have detected embryonic Hox-5.1 transcripts of ≈4.2, ≈2.6, and ≈1.4 kb. The two larger probably correspond to the 4.2- and 2.8- or 2.5-kb transcripts reported for the human gene (34), whereas a 1.4-kb transcript was detected for a *Hox-5.1*-related gene in the rat (34). Hox-5.1 transcripts were most abundant at day 10, the earliest time examined (data not shown). Similar to its human homolog, therefore, it is probably expressed by the end of neural tube closure (34), a stage when somite formation is well underway. Among adult tissues analyzed, only testis, kidney, intestine, and heart had detectable Hox-5.1 transcripts. Other murine homeogenes are expressed in testis and kidney of the adult, including *Hox-1.1* (10), *-1.2* (10), *-1.3* (42), *-2.3* (41), and -6.1 (43). By contrast, *Hox-1.4* (a *Hox-5.1*-related gene) is expressed exclusively in the adult testis (12, 14, 23). Homeogene expression in the intestine has also been observed for *Hox-1.6* (20), but no others have yet been reported for adult heart. Significantly, human Hox-5.1 transcripts are detected in human embryonic heart. By RNA gel blot analysis, Mavilio *et al.* (34) demonstrated human *Hox-5.1* expression in embryonic spinal cord and backbone rudiments. We have obtained parallel data with the murine *Hox-5.1* after *in situ* hybridization to mouse embryos. *Hox-5.1* is expressed in the spinal cord and prevertebrae. Homeogene expression in prevertebrae has been reported for *Hox-1.2* (44), *Hox-1.3*

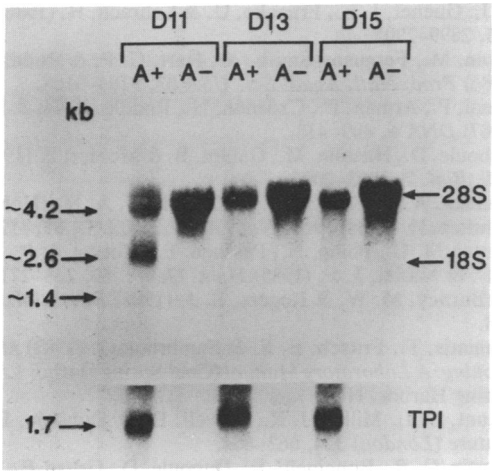

FIG. 4. RNA gel blot analysis of Hox-5.1 embryonic transcripts. Poly(A)$^+$ (lanes A+) and poly(A)$^-$ (lanes A−) RNA prepared from total RNA of embryos at days 11 (lanes D11), 13 (lanes D13), and 15 (lanes D15) were electrophoresed, transferred to nylon membranes, and hybridized with probe b (Fig. 1). Signals from transcripts of ≈4.2, ≈2.6, and ≈1.4 kb are indicated on the left. The signal from the intact 1.7-kb transcript for triose phosphate isomerase (TPI) is shown. The positions of 28S and 18S rRNA are given.

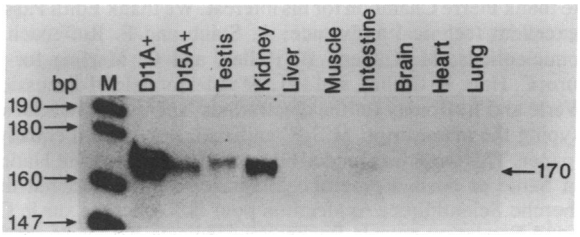

FIG. 5. S1 nuclease analysis of RNA from adult and embryonic tissues. Embryonic poly(A)$^+$ RNA from days 11 (lane D11A+) and 15 (lane D15A+) was used in S1 nuclease protection analysis in conjunction with poly(A)$^+$ RNA of eight adult tissues (identified by lane). Specific protection of the input probe was observed for samples from testis, kidney, heart, and intestine and was of the same size (170 bp) as for the embryonic RNA samples. Molecular size markers (lane M) are given at left.

(45), *Hox-3.1* (46), and *Hox-1.5* (S.J.G., unpublished observations). *Hox-5.1* is expressed anterior to all but *Hox-1.5*, which is detected in the first cervical prevertebrae (S.J.G., unpublished observations). The *Hox-5.1* expression boundary occurs at the second cervical prevertebra (C2), the anlagen for the axis. Further work should define the limits of expression in the spinal cord with respect to other reported homeogenes.

These results are in agreement with the suggestion of Gaunt *et al.* (32) for *Hox-1.5* (see also refs. 47 and 48) concerning its possible function as a positional cue along the developing body axis. Similarly, *Hox-5.1* could be one member of a family of regulatory genes (homeogenes) whose expression at different times and positions along the rostro–caudal axis would be required to establish a correct developmental plan. In this respect, it is interesting to note that a number of developmental mutants affecting the axial or appendicular skeleton have been mapped to mouse chromosome 2. Among them, the rachiterata (*rh*) mutation (49, 50) has been proposed (51) as a candidate for vertebrate "homeotic" transformation. Homozygous *rh/rh* neonates characteristically have six rather than seven cervical vertebrae due to an anterior shift of the cervico–thoracic boundary. Moreover, in all affected animals the axis is missing, reduced, or deformed by a supernumerary neural arch. Abnormal vertebral development can be detected by day 13 of gestation and malformation of the thoracic somites in *rh/rh* embryos has been observed as early as day 11 (49). Interestingly, these dysmorphic features correlate well with the location and time of *Hox-5.1* expression. *rh* has been genetically mapped between the fidget and nonagouti loci (50). *Hox-5.1* lies within band D of mouse chromosome 2 and so falls within this broad region as well. We have initiated experiments to determine whether these genes are related.

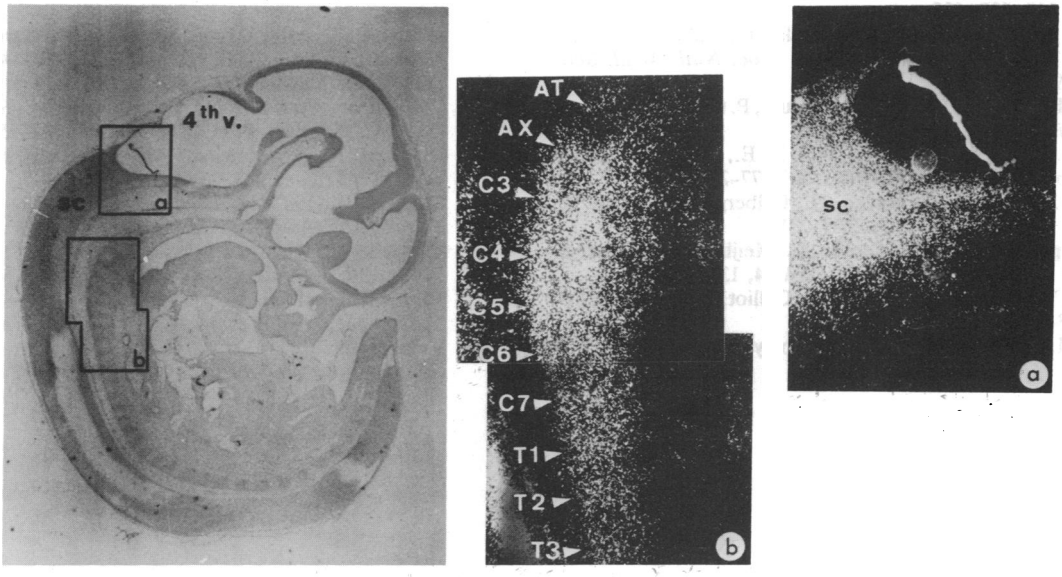

FIG. 6. *In situ* hybridization to day-12.5 mouse embryo. (*Left*) Light micrograph of sagittal section of day-12.5 mouse embryo used for *in situ* hybridization. Boxed areas a and b enclose regions of embryonic spinal cord and prevertebral column, respectively. The fourth ventrical (4th v.) of the developing hindbrain and the spinal cord (sc) are marked. (*Middle* and *Right*) Results of *in situ* hybridization with Hox-5.1 anti-sense ^{35}S-labeled probes. (*Right*) Darkfield illumination of boxed area a in light micrograph (*Left*). Silver grains are visualized as bright spots densely packed over the spinal cord (sc). (*Middle*) Darkfield illumination of prevertebral column shown in box b in light micrograph (*Left*). Prevertebrae for the atlas (AT), axis (AX), and subsequent cervical (C3–C7) and thoracic (T1–T3) prevertebrae are marked by arrowheads. Note the density of silver grains over the prevertebral axis, whereas the density over the atlas is at background level. Control hybridizations on parallel sections with Hox-5.1 sense probes gave no specific hybridization above background and, therefore, are not shown.

We thank Pierre Chambon for his interest. We thank Edith Passage for excellent technical assistance; A. Staub and F. Ruffenach for oligonucleotides; M. Gilbert, B. Heller, and C. Marfing for cell culture; C. Hart, B. Galliot, and J.-L. Mandel for helpful discussions; C. Werlé and B. Boulay for the illustrations; and the secretarial staff for typing the manuscript. M.S.F. and A.B. contributed equally to this paper. This work was funded by grants from the Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique, Association pour la Recherche sur le Cancer, and Fondation pour la Recherche Médicale Française. M.S.F. is a Postdoctoral Fellow of the Medical Research Council of Canada.

1. McGinnis, W., Garber, R. L., Wirz, J., Kuroiwa, A. & Gehring, W. J. (1984) *Cell* **37**, 403–408.
2. Scott, M. P. & Weiner, A. J. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4115–4119.
3. Gehring, W. J. (1987) *Science* **236**, 1245–1252.
4. McGinnis, W., Hart, C. P., Gehring, W. J. & Ruddle, F. H. (1984) *Cell* **38**, 675–680.
5. Levine, M., Rubin, G. M. & Tjian, R. (1984) *Cell* **38**, 667–673.
6. Colberg-Poley, A. M., Voss, S. D., Chowdhury, K. & Gruss, P. (1985) *Nature (London)* **314**, 713–718.
7. Jackson, I. J., Schofield, P. & Hogan, B. (1985) *Nature (London)* **317**, 745–748.
8. Hart, C. P., Awgulewitsch, A., Fainsod, A., McGinnis, W. & Ruddle, F. H. (1985) *Cell* **43**, 9–18.
9. Hauser, C. A., Joyner, A. L., Klein, R. D., Learned, T. K., Martin, G. R. & Tjian, R. (1985) *Cell* **43**, 19–28.
10. Colberg-Poley, A. M., Voss, S. D., Chowdhury, K., Stewart, C. L., Wagner, E. F. & Gruss, P. (1985) *Cell* **43**, 39–45.
11. Joyner, A. L., Kornberg, T., Coleman, K. G., Cox, D. R. & Martin, G. R. (1985) *Cell* **43**, 29–37.
12. Wolgemuth, D. J., Engelmyer, E., Duggal, R. N., Gizang-Ginsberg, E., Mutter, G. L., Ponzetto, C., Viviano, C. & Zakeri, Z. F. (1986) *EMBO J.* **5**, 1229–1235.
13. Awgulewitsch, A., Utset, M. F., Hart, C. P., McGinnis, W. & Ruddle, F. H. (1986) *Nature (London)* **320**, 328–335.
14. Rubin, M. R., Toth, L. E., Patel, M. D., d'Eustachio, P. & Nguyen-Huu, M. C. (1986) *Science* **233**, 663–667.
15. Shepherd, J. C. W., McGinnis, W., Carrasco, A. E., De Robertis, E. M. & Gehring, W. J. (1984) *Nature (London)* **310**, 70–71.
16. Laughon, A. & Scott, M. P. (1984) *Nature (London)* **310**, 25–31.
17. Desplan, C., Theis, J. & O'Farrell, P. H. (1985) *Nature (London)* **318**, 630–635.
18. Fainsod, A., Bogarad, L. D., Ruusala, T., Lubin, M., Crothers, D. M. & Ruddle, F. H. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9532–9536.
19. Kessel, M., Schulze, F., Fibi, M. & Gruss, P. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5306–5310.
20. Baron, A., Featherstone, M. S., Hill, R. E., Hall, A., Galliot, B. & Duboule, D. (1987) *EMBO J.* **6**, 2977–2986.
21. Breier, G., Bucan, M., Francke, U., Colberg-Poley, A. M. & Gruss, P. (1986) *EMBO J.* **5**, 2209–2215.
22. Deschamps, J., de Laaf, R., Joosen, L., Meijlink, F. & Destrée, O. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1304–1308.
23. Duboule, D., Baron, A., Mähl, P. & Galliot, B. (1986) *EMBO J.* **5**, 1973–1980.
24. Bucan, M., Yang-Feng, T., Colberg-Poley, A. M., Wolgemuth, D. J., Guenet, J. L., Francke, U. & Lehrach, H. (1986) *EMBO J.* **5**, 2899–2905.
25. Rabin, M., Ferguson-Smith, A., Hart, C. P. & Ruddle, F. H. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9104–9108.
26. Lonai, P., Arman, E., Czosnek, H., Ruddle, F. H. & Blatt, C. (1987) *DNA* **6**, 409–418.
27. Duboule, D., Haenlin, M., Galliot, B. & Mohier, E. (1987) *Mol. Cell. Biol.* **7**, 2003–2006.
28. Poutska, A., Rackwitz, M. R., Frischauf, A. M., Hohn, H. & Lehrach, H. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4129–4133.
29. Mattei, M. G., Philip, N., Passage, E., Moisan, J. P., Mandel, J. L. & Mattei, J. F. (1985) *Hum. Genet.* **69**, 268–271.
30. McBurney, M. W. & Rogers, B. J. (1982) *Dev. Biol.* **89**, 503–508.
31. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
32. Gaunt, S. J., Miller, J. R., Powell, D. & Duboule, D. (1986) *Nature (London)* **324**, 662–664.
33. Martin, G. R., Boncinelli, E., Duboule, D., Gruss, P., Jackson, I., Krumlauf, R., Lonai, P., McGinnis, W., Ruddle, F. & Wolgemuth, D. (1987) *Nature (London)* **325**, 21–22.
34. Mavilio, F., Simeone, A., Giampaolo, A., Faiella, A., Zappavigna, V., Acampora, D., Poiana, G., Russo, G., Peschle, C. & Boncinelli, E. (1986) *Nature (London)* **324**, 664–668.
35. Kozak, M. (1987) *Nucleic Acids Res.* **15**, 8125–8148.
36. Regulski, M., McGinnis, N., Chadwick, R. & McGinnis, W. (1987) *EMBO J.* **6**, 767–777.
37. Harvey, R. P., Tabin, C. J. & Melton, D. A. (1986) *EMBO J.* **5**, 1237–1244.
38. Hart, C. P., Fainsod, A. & Ruddle, F. H. (1987) *Genomics* **1**, 182–195.
39. Duboule, D., Galliot, B., Baron, A. & Featherstone, M. S. (1988) in *Cell to Cell Signals in Mammalian Development*, NATO Advanced Study Institute Series, eds. de Laat, S., Bluemink, J. G. & Mummery, C. L. (Springer, Berlin), in press.
40. Cannizzaro, L. A., Croce, C. M., Griffin, C. A., Simeone, A., Boncinelli, E. & Huebner, K. (1987) *Am. J. Hum. Genet.* **41**, 1–15.
41. Meijlink, F., de Laaf, R., Verrijzer, P., Destrée, O., Kroezen, V., Hilkens, J. & Deschamps, J. (1987) *Nucleic Acids Res.* **15**, 6773–6786.
42. Odenwald, W. F., Taylor, C. F., Palmer-Hill, F. J., Friedrich, V., Jr., Tani, M. & Lazzarini, R. A. (1987) *Genes Dev.* **1**, 482–496.
43. Sharpe, P. T., Miller, J. R., Evans, E. P., Burtenshaw, M. D. & Gaunt, S. J. (1988) *Development* **102**, 397–407.
44. Toth, L. E., Slawin, K. L., Pintar, J. E. & Nguyen-Huu, M. C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6790–6794.
45. Dony, C. & Gruss, P. (1987) *EMBO J.* **6**, 2965–2975.
46. Utset, M. F., Awgulewitsch, A., Ruddle, F. H. & McGinnis, W. (1987) *Science* **235**, 1379–1382.
47. Gaunt, S. J. (1987) *Development* **101**, 51–60.
48. Fainsod, A., Awgulewitsch, A. & Ruddle, F. H. (1987) *Dev. Biol.* **124**, 125–133.
49. Theiler, K., Varnum, D. & Stevens, L. C. (1974) *Z. Anat. Entwicklungsgesch.* **145**, 75–80.
50. Varnum, D. S. & Stevens, L. C. (1974) *J. Hered.* **65**, 91–93.
51. Gehring, W. J. (1985) *Cell* **40**, 3–5.