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Murine *Hox-1.7* Homeo-Box Gene: Cloning, Chromosomal Location, and Expression

MICHAEL R. RUBIN,[1,2] WALTER KING,[1,2] LESLIE E. TOTH,[1,2] IHOR S. SAWCZUK,[2] MICHAEL S. LEVINE,[3] PETER D'EUSTACHIO,[4] AND M. CHI NGUYEN-HUU[1,2]*

Department of Microbiology,[1] Department of Urology,[2] and Department of Biological Sciences,[3] Columbia University, New York, New York 10032, and Department of Biochemistry and Kaplan Cancer Center, New York University Medical School, New York, New York 10016 [4]

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A new murine homeo-box, called *Hox-1.7*, has been identified in a rare cDNA from F9 teratocarcinoma stem cells. The *Hox-1.7* homeo-box is 68 and 72% homologous to the *Drosophila* antennapedia (*Antp*) and *iab-7* homeo-boxes, respectively. A major 2.5-kilobase transcript and several minor transcripts were detected by Northern blot (RNA blot) analysis in adult tissues as well as in midgestational embryos. The posterior spinal cord was found to be a major site of *Hox-1.7* expression in 12.5-day-old embryos. Somatic cell hybrids were used to map the *Hox-1.7* gene to mouse chromosome 6. Restriction fragment length polymorphisms associated with either the *Hox-1.7* gene or the previously known *Hox-1* complex were identified. Their distribution patterns in recombinant inbred mouse strains were used to determine the linkage between the two loci as well as to other loci on chromosome 6. This maps *Hox-1* and *Hox-1.7* close to two mouse loci that affect morphogenesis, postaxial hemimelia (*px*) and hypodactyly (*Hd*).

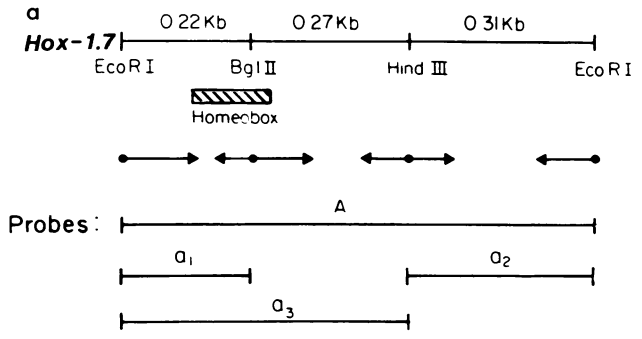
Homeotic and segmentation genes in *Drosophila* control cell determination and body pattern formation (29, 36, 37). Several such genes in the antennapedia (*Antp*), bithorax, and engrailed complexes contain a conserved 180-base-pair protein-coding sequence called the homeo-box (15, 35, 38, 41).

Genomic DNA from vertebrates, including mice and humans, contains several copies of homeo-boxes (28, 33, 34; reviewed in reference 30). In the mouse, 16 homeo-boxes have been identified. These define six genetic loci: *Hox-1*, *Hox-2*, *Hox-3*, *Hox-4*, *En-1*, and *En-2*, on mouse chromosomes 6, 11, 15, 12, 1, and 5, respectively (summarized in reference 31). All the homeo-boxes in these loci are expressed during embryogenesis (9, 14, 19, 20, 23–25, 32, 40, 44). Moreover, expression of several homeo-boxes has been shown to be localized to specific regions of the mouse embryo, analogous with the localized pattern of homeo-box gene expression in the *Drosophila* embryo (1, 2, 7, 13, 17, 18, 23, 26, 32, 39, 44, 50; L. E. Toth, K. L. Slawin, J. E. Pintar, and M. C. Nguyen-Huu, Proc. Natl. Acad. Sci. USA, in press). This expression pattern is entirely consistent with a role of homeo-box genes in murine morphogenesis, although direct genetic evidence for such a role is lacking. We report here the identification, chromosomal location, and expression pattern of a new murine homeo-box gene designated *Hox-1.7*.

Isolation and sequence analysis of the *Hox-1.7* cDNA. Embryonal carcinoma cells (45), the pluripotent stem cells of malignant teratocarcinomas, are developmentally similar to primitive ectodermal cells of the early mouse embryo and represent a useful model for studying certain aspects of early mammalian embryogenesis. A sensitive way to determine whether homeo-box sequences are expressed in embryonal carcinoma cells would be to construct and screen a large cDNA library from embryonal carcinoma cells for clones containing these sequences. We used the procedure of Huynh et al. (22) to prepare a cDNA library in the lambda bacteriophage vector gt-10 from poly(A)$^+$ RNA isolated from F9 embryonal carcinoma stem cells (4). A library of 10^6 cDNA clones was obtained from 10 μg of RNA. To screen for homeo-box-containing clones, the 1.7-kilobase (kb) *Hin*dIII fragment and the 2.1-kb *Eco*RI fragment that contain the human *Hu-1* and *Hu-2* homeo-boxes (28) were used as mixed probes for in situ plaque hybridization under reduced-stringency conditions as described by McGinnis et al. (33). We isolated seven positive clones after screening an amplified library of 5×10^5 cDNAs. DNA from these clones was analyzed by digestion with *Eco*RI and blot hybridization to each of the human *Hu-1* and *Hu-2* or the *Drosophila Antp* (16) and ultrabithorax (*Ubx*) (3) homeo-box probes. One clone contained a 3-kb insert which hybridized only to the *Hu-1* probe. The other six clones all contained an *Eco*RI insert of 0.8 kb that hybridized to each of the *Hu-1*, *Hu-2*, and *Ubx* probes but not to the *Antp* probe (data not shown). These clones, originally designated MH-1 and later renamed *Hox-1.7* to conform to current nomenclature (31), were chosen for further analysis.

Figure 1a shows the restriction map of the cDNA clone *Hox-1.7*, and Fig. 1b shows the sequence of the 0.8-kb *Eco*RI insert determined by the chain termination method. The DNA contains in one orientation an open reading frame which includes a homeo-box. Table 1 shows a sequence comparison of the *Hox-1.7* homeo-box with other *Drosophila*, human, and mouse homeo-boxes. Although the cloned cDNA does not represent a complete copy of the mRNA, it contains the complete homeo-box. A termination codon was found 7 amino acids downstream from the homeo-box, and no initiation codon was found in the 25 amino acids preceding it. Although the sequence at the 5′ *Eco*RI site is identical to the sequence of the linker used in cDNA cloning, the sequence at the 3′ *Eco*RI site is not. Therefore, the 0.8-kb cDNA lacks 5′ untranslated and coding sequences as well as some 3′ untranslated sequences. Restriction mapping and Southern blotting experiments were used to position the cDNA clone on the *Hox-1.7* genomic map (see Fig. 4a) (data not shown). Sequences between the *Bgl*II site and the 3′ *Eco*RI site of the cDNA were found in

* Corresponding author.



b

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-9                                15                  30                  45
AATTCCGCG  GAG AAT GAG AGC CGC GGA GCA AGC CCC CCG ATC GAT CCC AAT AAC
           Glu Asn Glu Ser Arg Gly Ala Ser Pro Pro Ile Asp Pro Asn Asn

                  60                  75                  90
CCG GCT GCC AAC TGG CTA CAT GCT CGC TCC ACT CGG AAG AAG CGA TGC CCC TAC
Pro Ala Ala Asn Trp Leu His Ala Arg Ser Thr Arg Lys Lys Arg Cys Pro Tyr

    105                 120                 135                 150
ACA AAA CAC CAG ACG CTG GAA CTG GAG AAG GAG TTT CTG TTT AAC ATG TAC CTC
Thr Lys His Gln Thr Leu Glu Leu Glu Lys Glu Phe Leu Phe Asn Met Tyr Leu

            165                 180                 195
ACA CGG GAC CGC AGG TAC GAG GTG GCC CGG CTG CTC AAC CTC ACC GAA AGG CAG
Thr Arg Asp Arg Arg Tyr Glu Val Ala Arg Leu Leu Asn Leu Thr Glu Arg Gln

210  BglII          225                 240                 255
GTC AAG ATC TGG TTC CAG AAC CGC AGG ATG AAA ATG AAG AAA ATC AAC AAG GAC
Val Lys Ile Trp Phe Gln Asn Arg Arg Met Lys Met Lys Lys Ile Asn Lys Asp

        270                 290         300         310
CGA GCA AAA GAC GAG TGA G  CCTTTTAGGG  GCTCATTTAA  AAAGAGAGCA
Arg Ala Lys Asp Glu  *

       320         330         340         350         360         370
AGCTAGACAA  GAAAAAGAAA  GGACTGTCCG  TCTCCCTCTG  TCTTCCTCTC  CCCCAAACCC

       380         390         400         410         420         430
AGCCTCCACC  CGCACAAAGG  GGCTCTAAAT  CCCAGGCCTC  ATCTCCCCAC  TGGCAGTCCG

       440         450         460         470         480 HindIII 490
TGCTCAGGCT  GGCTCTTAGG  CCTGCGGCTT  TGATGGAGGA  GGTATTGTAA  GCTTCAGATA

       500         510         520         530         540         550
GAAAAAACAG  CTTCCTGTGA  CAGAAGGTTG  GGAATAAGCT  GGTTGACCGT  TCCACCAGCT

       560         570         580         590         600         610
GCGGAATAGC  GGGTGGGTTT  GTCGCGCCTG  AGGTTCGCGT  CCAGTAAAAA  AGGCAATAAG

       620         630         640         650         660         670
TCCATAAATA  ATCCAGATGG  CATAAGCTAA  TAATACATAC  ACAACGGAAA  GCGGCGTAAA

       680         690         700         710         720         730
GGCACCAACA  ACCAGCACGA  TGAACCAGGC  GCTAAAGCTC  CAATAACACA  AACTACGCCG

       740         750         760         770         780    786
GACCCAGATA  AGCGGAAACA  TTGCCAACAC  CACTCGAGTG  CGGCCCGGTG  GTGATG
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FIG. 1. (a) Restriction map of the *Hox-1.7* cDNA clone. The homeo-box location and the fragments used as hybridization probes are shown. The various restriction fragments were subcloned directionally into M13mp18 and M13mp19 vectors and sequenced by the chain termination method as previously described (40) (represented by the solid circles attached to arrows). The majority of the sequence was obtained for both strands. (b) DNA sequence of the 0.8-kb *EcoRI* fragment of the *Hox-1.7* cDNA clone. The conceptual translation is shown, and the 180-base-pair homeo-box region is underlined.

a contiguous arrangement at the very 3′ end of the 5.5-kb *Eco*RI genomic fragment of clone MH-1G33b. Sequences between the *Bgl*II site and the 5′ *Eco*RI site of the cDNA were not contiguous in the genomic DNA, indicating the presence of a splice site in this region.

Expression of the *Hox-1.7* gene. To gain some insight into the possible functions of the *Hox-1.7* gene, we used the *Hox-1.7* cDNA clone as a probe to analyze the expression of this gene in teratocarcinoma cells, mouse embryos, and adult mouse tissues. Since the mouse genome may contain sequences that cross-hybridize to the *Hox-1.7* homeo-box, it was important to define conditions under which this probe will hybridize specifically only to the *Hox-1.7* gene. Under stringent hybridization conditions, the cDNA probe detected a single 5.5-kb *Eco*RI fragment, an 8.5-kb *Bam*HI fragment, and two *Hin*dIII fragments of 0.8 and 0.7 kb in Southern blots of mouse genomic DNA (Fig. 2).

Figure 3 shows the hybridization, under stringent conditions, of the *Hox-1.7* cDNA probe to Northern blots (RNA blots) containing RNA isolated from various sources. No hybridization was detected with poly(A)$^+$ RNA from F9 stem cells (Fig. 3a, lane F9) and F9-derived parietal endoderm cells (lane F9D). *Hox-1.7* transcripts were found in mouse kidney tissue (lane KID). A major RNA species of 2.5 kb and two minor RNA species of 1.9 and 3.9 kb were clearly observed. Similarly sized transcripts were found in other adult tissues such as spinal cord, heart, and spleen, but not brain or uterus (data not shown). The integrity of the RNA present on the blots was confirmed by hybridization to an actin probe. Since *Hox-1.7* transcripts could be detected in F9 teratocarcinoma cells by the more sensitive technique of cDNA cloning but not by Northern hybridization, it appears that the *Hox-1.7* gene is expressed at very low levels in F9 teratocarcinoma cells. Such a low level of expression is consistent with the frequency at which *Hox-1.7* clones were found in the F9 cDNA library.

Figure 3b shows hybridization to poly(A)$^+$ RNA isolated from mouse embryos at days 11.5, 12.5, 13.5, 14.5, and 16.5 of gestation (lanes 1 to 5, respectively). The 2.5- and 3.9-kb embryonic transcripts appear identical in size to two of the transcripts found in adult kidney tissue. The level of these transcripts was relatively high at days 11.5 and 12.5 (lanes 1 and 2, respectively); it then decreased until it was undetectable on day 16.5 (lane 5). The integrity of the RNA present on the blots was confirmed by hybridization to a Harvey-*ras* probe.

Figure 3c shows hybridization to total RNA isolated from dissected parts of 12.5-day-old mouse embryos. Embryos were microdissected into four fractions: brain, anterior spinal cord, posterior spinal cord, and carcass. The brain and anterior spinal cord fractions were separated at the level of the pontine flexure. The anterior and posterior spinal cord

TABLE 1. Sequence comparison of *Hox-1.7* with other homeo-boxes

Homeo-box	% Homology		Reference(s)
	Nucleotides	Amino acids	
D. melanogaster			
iab-7	64	72	39
Antp	59	68	16, 33, 35, 41
en	49	42	15, 38
Human			
Hu-1	64	67	28
Hu-2	63	67	28
Mouse			
Hox-1.1	66	67	8
Hox-1.2	63	70	9
Hox-1.3	61	67	M. Patel and M.-C. Nguyen-Huu, unpublished data
Hox-1.4	65	65	14, 40
Hox-1.5	64	63	34
Hox-1.6	62	60	D. Duboule, personal communication
Hox-2.1	63	67	20, 23, 26
Hox-3.1	61	70	1, 5
En-1	54	43	24, 25
En-2	55	43	25

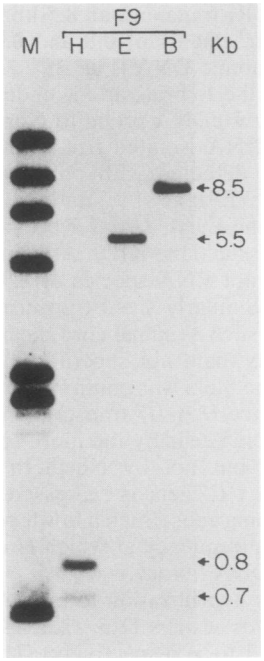
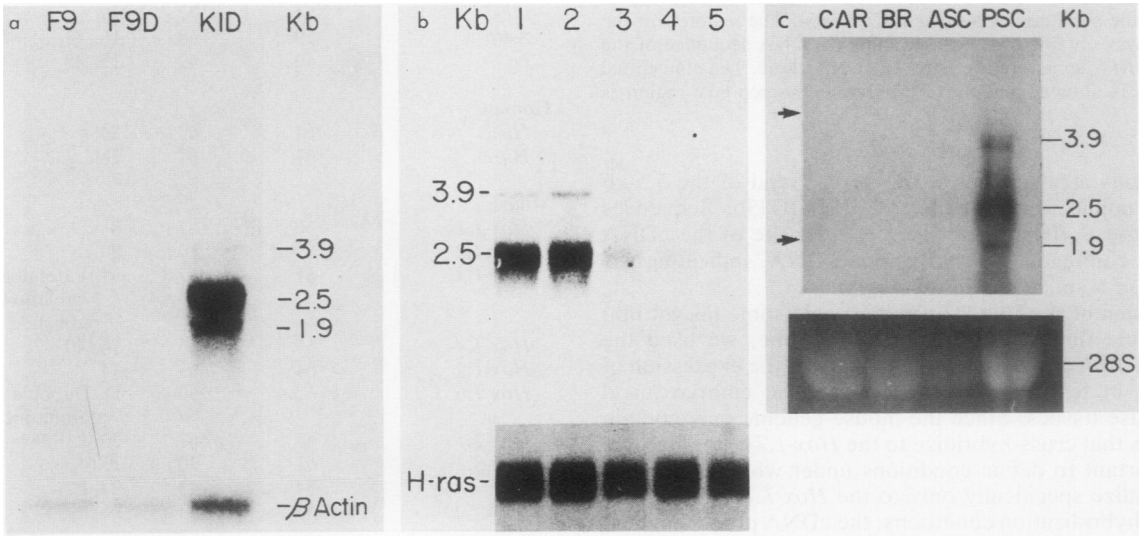


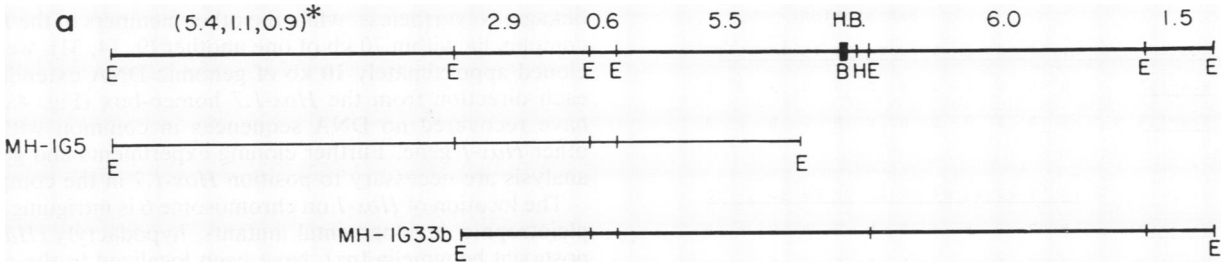
FIG. 2. *Hox-1.7* hybridization to unique genomic fragments. Hybridization of the *Hox-1.7* probe A (Fig. 1a) to a Southern blot containing murine (F9 cell) DNA cleaved with *Hin*dIII (lane H), *Eco*RI (lane E), or *Bam*HI (lane B). *Hin*dIII-digested lambda DNA fragments serve as molecular weight markers (lane M). Digested DNA (10 μg) was fractionated by electrophoresis on a 1.0% agarose gel, blotted onto nitrocellulose filter paper, and hybridized for 18 h at 68°C with nick-translated DNA (10 ng/ml; 2×10^8 cpm/μg) as previously described (40). The filter was washed to a final stringency of 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate at 68°C. Blots were exposed for 5 days at −70°C with an intensifying screen. The approximate sizes of the hybridizing genomic bands are indicated to the right in kilobases.

fractions were separated at the level of the second thoracic vertebra. The carcass fraction included the remainder of the embryo proper. A major 2.5-kb transcript and two minor transcripts, 1.9 and 3.9 kb, were detected at high levels in the embryonic posterior spinal cord (Fig. 3c, lane PSC). Upon longer exposures, lower levels of these three transcripts were found in embryonic anterior spinal cord and carcass fractions but not in the embryonic brain fraction (data not shown).

Chromosomal location of the *Hox-1.7* gene. To map the *Hox-1.7* gene to a mouse chromosome, we used Southern blotting to analyze a panel of somatic cell hybrids carrying various combinations of mouse chromosomes (11). Following *Eco*RI digestion, the *Hox-1.7* cDNA hybridized to a 5.5-kb band in mouse DNA and a smaller, distinct band in hamster DNA. The 5.5-kb hybridizing mouse DNA fragment was found only in the four hybrid cell lines that retained mouse chromosome 6 and not in the other cell lines (data not shown). Therefore the *Hox-1.7* gene maps to mouse chromosome 6.

To position *Hox-1.7* on the linkage map of chromosome 6, we searched for restriction fragment length polymorphisms associated with the gene. For these studies, two overlapping mouse genomic lambda clones which hybridized at high stringency to the *Hox-1.7* cDNA were isolated from a C57BL/6J DNA library. The two inserts, one 14.9 kb (clone MH-1G5) and the other 16.4 kb (clone MH-1G33b) in length, encompass a 23.9-kb genomic region roughly centered on the *Hox-1.7* homeo-box (Fig. 4a). *Eco*RI-digested DNA from various inbred strains of mice yielded two alternative band patterns when hybridized with the MH-1G5 genomic probe (Fig. 4b). To localize the *Hox-1.7* sequence on chromosome 6, we monitored inheritance of this polymorphism in 36 recombinant inbred strains of mice. These 36 recombinant inbred strains and 7 additional ones were also typed for inheritance of a polymorphism associated with the *Hox-1.4* sequence, a previously defined member of the *Hox-1* gene complex (14, 40, 51). Alleles at *Hox-1.7* and *Hox-1.4* were inherited concordantly in all 36 strains (Fig. 5), indicating with 95% confidence that the two loci are less than 2.9 centimorgans (cM) apart (43). Comparison of the strain distribution pattern observed for these markers with ones previously determined for other markers of chromosome 6 showed linkage between *Hox-1* and *Ggc* (gamma-glutamyl cyclotransferase) (49), *Igk* (immunoglobulin kappa chains) (12), and *Lyt-2* (lymphocyte antigen 2) (49). Use of the observed fractions of recombinant strains to estimate dis-





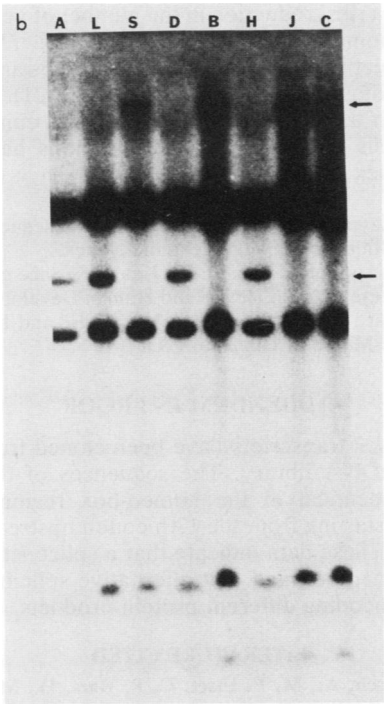


FIG. 4. Detection of a restriction fragment length polymorphism among inbred mouse strains by using the *Hox-1.7* genomic clone MH-1G5. (a) Restriction map of the *Hox-1.7* genomic region. The overlapping genomic clones, MH-1G5 and MH-1G33b, are shown below the map. The *Eco*RI sites are indicated (E). The terminal *Eco*RI sites are derived from the lambda vector. The approximate sizes of the *Eco*RI fragments are indicated. The locations of the *Eco*RI sites which separate the 0.9-, 1.1-, and 5.4-kb fragments in MH-1G5 have not been determined, as indicated by the asterisk. The black box represents the *Hox-1.7* homeo box (H.B.). The *Bgl*II (B) and *Hind*III (H) sites located at the 3' end of the 5.5-kb fragment correspond to sites in the cDNA. (b) A restriction fragment length polymorphism among inbred mouse strains is associated with the *Hox-1.7* genomic clone MH-1G5. Hybridization of the MH-1G5 genomic clone to a Southern blot (as described in the legend to Fig. 2) containing *Eco*RI-restricted DNA isolated from inbred mouse strains AKR/J (lane A), C57L/J (lane L), SWR/J (lane S), DBA/2J (lane D), C57BL/6J (lane B), C3H/HeJ (lane H), SJL/J (lane J), and BALB/cJ (lane C). The two polymorphic restriction fragments are indicated by arrows.

tance among the loci yielded the following map: *Hox-1*-1.9 cM-*Ggc*-2.6 cM-*Igk*, *Ly-2*. Comparison of the *Hox-1* strain distribution pattern with ones determined for 274 loci mapped elsewhere in the genome revealed no correlations better than expected by chance.

Conclusion. The data reported here define a new mouse homeo-box-containing gene, *Hox-1.7*, and provide some information about its chromosomal location, tissue-specific expression, and developmental regulation.

Sequence analysis of the *Hox-1.7* cDNA shows that the homeo-box is located at the carboxyl end of the deduced protein sequence, a location similar to that of *Drosophila* homeo-boxes within the various *Drosophila* homeotic and segmentation genes (3, 16, 27, 41). Among all the known *Drosophila*, human, and mouse homeo-boxes, the highest homology to *Hox-1.7* is found with the *Drosophila iab-7* homeo-box (39). The significance of this observation is unclear at present.

We do not know when the *Hox-1.7* gene is first expressed in embryogenesis. The very low level of expression in F9 teratocarcinoma cells, as detected by cDNA cloning, raises the possibility that the *Hox-1.7* gene is also expressed at a low level in the inner cell mass or the primitive ectoderm cells of preimplantation embryos. Unlike many other genes in the *Hox-1*, *Hox-2*, *Hox-3*, *En-1*, and *En-2* complexes, whose transcripts are induced during retinoic acid treatment of teratocarcinoma cells (5, 8–10, 20, 24, 25, 48), *Hox-1.7* transcripts remain undetectable by Northern blot analysis

FIG. 3. Expression of the *Hox-1.7* gene in teratocarcinoma cells, adult mouse tissues, and mouse embryos. (a) Northern blot containing 20 μg of poly(A)$^+$ RNA isolated from F9 stem cells (lane F9); F9-derived parietal endoderm cells (F9 cells were induced to differentiate for 4 days in the presence of 5×10^{-7} M retinoic acid and 5×10^{-4} M dibutyrylcyclic AMP [46]; these differentiated F9 cells were stage-specific embryonic antigen I negative and expressed high levels of collagen type IV mRNA) (lane F9D); and adult mouse kidney (lane KID). (b) Northern blot containing 10 μg of poly(A)$^+$ RNA from ICR mouse embryos isolated at days 11.5 (lane 1), 12.5 (lane 2), 13.5 (lane 3), 14.5 (lane 4), and 16.5 (lane 5) of gestation. (c) Northern blot containing 30 μg of total RNA isolated from microdissected fractions of 12.5-day ICR mouse embryos: carcass (lane CAR); brain (anterior to the level of the pontine flexure) (lane BR); anterior spinal cord (from the level of the pontine flexure to the level of the second thoracic vertebra) (lane ASC); and posterior spinal cord (posterior to the second thoracic vertebra) (lane PSC). After isolation and quantitation, RNA was electropheresed on 1.2% agarose gels containing formaldehyde and blotted onto nitrocellulose filters. The 18S and 28S rRNAs served as molecular weight markers. Blots were hybridized to probes prepared by nick translation of the A, a1, or a2 fragment of clone *Hox-1.7*. Blots were hybridized at 42°C for 12 to 16 h as previously described (40). Blots were washed with 0.1× SSC–0.1% sodium dodecyl sulfate at 68°C and exposed for 3 days at −70°C with an intensifying screen. The approximate sizes of the transcripts are shown in kilobases. Identical results were found with subfragments a1 and a2 (Fig. 1a) as probes. Probe a1 contains 221 nucleotides, 137 of which are *Hox-1.7* homeo-box sequences, and probe a2 contains 307 nucleotides of *Hox-1.7* 3' untranslated sequences. The panel below each autoradiogram confirms that equal amounts of RNA were present in the lanes as determined by rehybridization to actin (panel a) or Harvey-*ras* (panel b) probes or the 28S rRNA band after ethidium bromide staining (panel c).

BxD strains:

Locus	1	2	5	6	8	9	11	12	13	14	15	16
Hox-1.7	B	B	B	D	D	B	D	B	D	B	B	B
Hox-1.4	B	B	B	D	D	B	D	B	D	B	B	B

	18	19	22	23	24	25	27	28	29	30	31	32
Hox-1.7	B	D	D	B	B	D	D	D	B	D	B	B
Hox-1.4	B	D	D	B	B	D	D	D	B	D	B	B

BxH strains:

	2	3	4	6	7	8	9	10	11	12	14	19
Hox-1.7	H	B	B	B	B	B	B	H	B	B	B	B
Hox-1.4	H	B	B	B	B	B	B	H	B	B	B	B

CxB strains:

	D	E	G	H	I	J	K
Hox.1.7	-	-	-	-	-	-	-
Hox 1.4	B	B	C	B	C	C	C

FIG. 5. Inheritance of *Hox-1.7* and *Hox-1.4* in recombinant inbred mouse strains. Recombinant inbred strains of mice were derived by inbreeding pairs of F2 hybrid mice from a cross between two inbred progenitor strains as follows: B×D, C57BL/6J × DBA/2J; B×H, C57BL/6J × C3H/HeJ; C×B, BALB/cBy × C57BL/6By (47). Mice (obtained from Jackson Laboratory, Bar Harbor, Maine) were typed for DNA polymorphism at *Hox-1.7* (Fig. 4b). All strains were homozygous for one of the progenitor strain forms of the locus, as indicated by the letters B (C57BL/6), C (BALB/c), D (DBA/2), and H (C3H/He).

and RNase protection assay (Fig. 3 and data not shown). *Hox-1.7* expression was relatively high at days 11.5 and 12.5 of gestation and declined afterwards. This pattern of temporal expression seems to be characteristic of all *Hox-1* genes (9, 14, 40). In contract, all of the homeo-box genes in the *Hox-2* and *Hox-3* complexes analyzed so far seem to be expressed continuously throughout later stages of embryogenesis (1, 19, 20, 23, 26).

With respect to tissue specificity in the midgestational embryo, the expression of *Hox-1.7* both resembles and differs from that of other homeo-box genes. *Hox-1.7* is similar to many other homeo-box genes with respect to its high level of expression in the embryonic and adult spinal cord (17, 26, 50; Toth et al., in press). However, within the embryonic spinal cord, *Hox-1.7* is preferentially expressed in the posterior region, i.e., below the level of the second thoracic vertebra. In contrast, the other homeo-boxes analyzed so far are all expressed in more anterior regions of the spinal cord (17, 26, 50; Toth et al., in press). Thus the different murine homeo-boxes seem to be expressed at different rostrocaudal regions of the spinal cord. This region-specific expression is strikingly similar to that of the different *Drosophila* homeo-box genes in the ventral nerve cords of *Drosophila* embryos and larvae.

By the criteria of its sequence homology to the *Drosophila Antp* homeo-box (Table 1) and its genetic linkage (Fig. 5), *Hox-1.7* is a member of the *Hox-1* complex. The designation *Hox-1.7* is in accord with the provisional scheme for the nomenclature of mouse homeo-box genes (31). At the same time, its physical relationship to the rest of the cluster is unclear. Analysis of recombinant inbred mice indicates tight linkage. Nevertheless, while the other members of the *Hox-1* complex lie within 70 kb of one another (9, 14, 31), we have cloned approximately 10 kb of genomic DNA extending in each direction from the *Hox-1.7* homeo-box (Fig. 4a) and have recovered no DNA sequences in common with any other *Hox-1* gene. Further cloning experiments and genetic analysis are necessary to position *Hox-1.7* in the complex.

The location of *Hox-1* on chromosome 6 is intriguing. Two pleiotrophic developmental mutants, hypodactyly (*Hd*) and postaxial hemimelia (*px*), have been localized to the region of chromosome 6 near *Igk* (12, 21, 42). *px* results in limb defects, extra ribs, reduction in the number of vertebrae, and sterility in homozygotes of both sexes (42). *Hd* results in single-digit feet and greatly reduced forelimbs and hindlimbs in heterozygotes and death in homozygotes (21). Our results, together with those of Bućan et al. (6), are consistent with the hypothesis that one or both mutations lie within the *Hox-1* complex.

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ADDENDUM IN PROOF

Two *Hox-1.7* transcripts have been cloned from a guinea pig kidney cDNA library. The sequences of these cDNA clones are identical in the homeo-box region but differ significantly starting from the 12th codon upstream from the homeo-box. These data indicate that a splice site is present 5′ to the homeo-box and that alternative splicing results in transcripts encoding different protein products.

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