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A Mouse Homeo Box Gene, *Hox-1.5*, and the Morphological Locus, *Hd*, Map to Within 1 cM on Chromosome *6*

Beverly A. Mock, Lawrence A. D'Hoostelaere, Roberta Matthai and Konrad Huppi

Laboratory of Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

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ABSTRACT

Mo-10, a homeo box-containing sequence in the *Hox-1* complex of genes referred to as *Hox-1.5*, was found to be polymorphic in inbred and wild mice, and a strain distribution of three allelic forms of *Hox-1.5* are reported. The position of *Hox-1.5* was mapped in backcross experiments to within 1 cM of the hypodactyly locus on chromosome *6*. This identifies the *Hd* mutation as a useful model for the examination of homeo box expression during mammalian development.

ADVANCES in the understanding of genes controlling mammalian development have been fostered by the discovery of a highly conserved coding sequence, the homeo box. Homeotic gene complexes, composed of clusters of homeo box sequences, were first described in Drosophila where they possess regulatory functions in controlling segmentation and other morphogenetic processes along the anterior-posterior axis of developing embryos (GARCIA-BELLIDO 1975; LEVINE, RUBIN and TJIAN 1984; LEWIS 1974; MANLEY and LEVINE 1985). These 180-bp sequences have a high degree of homology with sequences in vertebrates (CARRASCO *et al.* 1984; LEVINE, RUBIN and TJIAN 1984; MCGINNIS *et al.* 1984a), and they share homologies with sequences of DNA binding proteins that control developmental processes in bacteria and yeast (LAUGHON and SCOTT 1984; OHLENDORF, ANDERSON and MATTHEWS 1983; PABO and SAUER 1984; SHEPHERD *et al.* 1984). Since clusters of homeo boxes are differentially expressed during murine embryogenesis, it has been suggested that they play a role in controlling mammalian development (COLBERG-POLEY *et al.* 1985; MANLEY and LEVINE 1985).

Clusters of homeo box genes are dispersed among Drosophila chromosomes; recent studies have found similarities in the dispersion of homeo box genes among mouse chromosomes. In mice, two classes of homeo box sequences have been associated with at least four different linkage groups. The engrailed class has been mapped to chromosome *1* (JOYNER *et al.* 1985a) and sequences, referred to as *Hox-1*, *Hox-2* and *Hox-3* loci, belonging to the Antennapedia class have been mapped to chromosomes *6*, *11* and *15*, respectively (AWGULEWITSCH *et al.* 1986; BUCAN *et al.* 1986; COLBERG-POLEY *et al.* 1985; HART *et al.* 1985; HAUSER *et al.* 1985; JOYNER *et al.* 1985b; MCGINNIS *et al.* 1984b; RABIN *et al.* 1985, 1986; RUBIN *et al.* 1986).

Since homeo boxes are associated with the timing and expression of segmental development in Drosophila, it is hypothesized that they are linked to murine loci known to influence morphogenesis. One of these homeotic sequences, *Hox-1.5* (MARTIN *et al.* 1987) [also referred to as *Hox 1-4* (DUBOULE *et al.* 1986) and *Mox 6.5* (GRUSS and KESSEL 1986)], is within the *Hox-1* complex of genes that contains 6–10 homeo box sequences located proximal to the immunoglobulin κ light chain (*Igk*) on mouse chromosome *6* (MCGINNIS *et al.* 1984b) and more recently found to be distal to the T cell receptor β chain (*Tcrb*) (BUCAN *et al.* 1986).

We examined the hypothesis that *Hox-1.5* was allelic with the *Hd* and wild-type (+) alleles of the hypodactyly locus on mouse chromosome *6*. The hypodactyly mutation is known to be lethal in the homozygous state; mice heterozygous for *Hd* exhibit a shortening of the first digit of the hind feet and occasionally this heterozygous condition is also lethal (HUMMEL 1970). Since multiple markers between *Tcrb* and waved-1 (*wa-1*), including *Hd*, on chromosome *6* are easily followed in genetic crosses, we examined the cosegregation of these loci in ongoing backcross experiments. As a result, *Hox-1.5* alleles were determined for a variety of inbred, recombinant inbred and wild mice.

MATERIALS AND METHODS

Mice: Inbred strains of mice were variously obtained from The Jackson Laboratory, Bar Harbor, ME (A/J, ABP/Le, BALB/cByJ, BUB/BnJ, C3H/HeJ, C57BL/6J, C57BL/6ByJ, C58/J, DBA/2J, I/LnJ, MRL/MpJ-*lpr*, NZB/BlNJ, P/J, PL/J, SWR/J, YBR/Ei), the National Institutes of Health, Division of Natural Resources, Bethesda, Maryland (AKR/N, C57BL/10N, CBA/N, NZW/N), J. HILGERS, the Netherlands Cancer Institute, Amsterdam (BALB/cHeA, 020/A, STS/A and the CXS and OXA recombinant inbred strains), M. NESBITT, University of California at San Diego, La Jolla, California (AXB and BXA recombinant inbred

strains), M. POTTER, NCI Contract N01-CB2-5584 at Hazleton Laboratories, Rockville, Maryland (BALB/cAnPt, C3H/HeN, C57L/J, C.B6.C3-*Hd*/+, SJL/JLwPt, CXB recombinant inbred strains, wild mouse strains), D. GIBSON, University of Sherbrooke, Sherbrooke, Quebec (NAK, C58.B6.C3-*Hd*/+) and R. J. BERRY, University of London, London (wild mouse tissues from Scotland).

C.B6.C3-*Hd*/+ congenic mice were derived by the introgressive backcrossing of the *Hd* marker from B6.C3-*a/a*, Va^J/+, *Hd*/+ congenic mice onto the BALB/cAnPt background (D'HOOSTELAERE *et al.* 1985). C58.B6.C3-*Hd*/+ congenic mice were similarly derived in backcrosses of the *Hd* marker onto the C58/J background (D. GIBSON, personal communication). The NAK mouse, an *Igk* recombinant, was originally derived by backcrossing (NZB × AKR)F_1 females to NZB males (GIBSON *et al.* 1984). AKR and NZB mice have been shown to carry the *a* and *b* haplotypes, respectively, for *Igk* by both isoelectric focusing patterns (*Igk-Ef1*, *Igk-Ef2*) and restriction fragment length polymorphisms (all *Igk-V* region probes examined). The NAK mouse is homozygous for select *Igk* genes and defines a recombination site within the variable (V) region [(*Igk-Ef2*^{b}; *Igk-V24*^{b}; *Igk-V11*^{b}; *Igk-V9*^{b}) (*Igk-V10*^{a}; *Igk-V8*^{a}; *Igk-V4*^{a}; *Rn7s-6*^{a}; *Igk-Ef1*^{a}; *Igk-V21*^{a}; *Igk-J*^{a}; *Ly2*^{a}; *Ly3*^{a})] (BOYD, GOLDRICK and GOTTLIEB 1986; D'HOOSTELAERE and GIBSON 1986; GIBSON *et al.* 1984; GOLDRICK *et al.* 1985; TAYLOR *et al.* 1985).

Genetic crosses: In one backcross experiment SJL/LwPt mice were mated with C.B6.C3-*Hd*/+ mice, and male progeny which expressed the hypodactyly (*Hd*) morphological marker were mated with SJL/LwPt. The resultant 88 N2 progeny were typed for *Tcrb* and *Igk* alleles (D'HOOSTELAERE, JOUVIN-MARCHE and HUPPI 1985); 84 of these 88 mice were typed for *Hox-1.5* alleles. In a second backcross experiment, C58.B6.C3-*Hd*/+ mice were mated with ABP/Le *wa-1/wa-1* mice and the *Hd*/+ male mice were backcrossed to ABP/Le. Recombinants between *Hd* and *wa-1* were selected for further typing of loci based on the presence or absence of both markers in the same mouse (Figure 1).

Data analysis: A Bayesian statistical approach (SILVER and BUCKLER 1985; SILVER 1985) was used to determine the most likely position of the test locus for data on recombinant inbred strains. Maximum likelihood estimates of recombination probabilities ($\hat{c}$), their variances ($v_{\hat{c}}$) and standard errors ($s_{\hat{c}}$) were calculated according to GREEN (1981): $\hat{c} = r/n$, $v_{\hat{c}} = \hat{c}(1 - \hat{c})/n$, $s_{\hat{c}} = \sqrt{v_{\hat{c}}}$, *where r* is the number of recombinations in a sample of size *n*.

Gel electrophoresis, Southern blotting and hybridization: High molecular weight DNAs prepared from mouse livers or kidneys were digested for 4–6 hr with restriction endonucleases (Boehringer Mannheim) and run on 0.7% horizontal agarose gels at voltage gradients of approximately 1 V/cm in a 40 mM Tris-acetate (pH 7.4), 20 mM sodium acetate, 1 mM EDTA running buffer. After staining with ethidium bromide and photography under UV light, gels were treated with 0.25 N HCl for 7 min, denatured with 0.5 N NaOH, 1 M NaCl for 30 min, and subsequently neutralized in 0.5 M Tris-HCl (pH 7.2), 3 M NaCl for 1 hr (HUPPI *et al.* 1985). DNA was transferred to nitrocellulose and blots were hybridized with probes for 16 hr. The probes were labeled with ^{32}P-dCTP by nick-translation to specific activities of 1–2 × 10^8 cpm/μg. After hybridization, filters were rinsed in 3× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate), washed in 1× SSC at 65° for 5 min, and subsequently washed for 30 min in 0.2× SSC. Hybridized filters were exposed to film from 1 to 3 days.

Probes: The 1.4-kilobase pair (kb) *Eco*RI insert of pM0-10 which recognizes the *Hox-1.5* locus was kindly provided by W. MCGINNIS (MCGINNIS *et al.* 1984b). The 86T1 cDNA clone of the T-cell receptor β chain (*Tcrb*) was obtained from M. DAVIS (HEDRICK *et al.* 1984). A *Bam*HI-*Eco*RI insert was isolated from this clone and used for hybridization in these studies. This insert extends from the entire constant, joining, and diversity region to codon 100 in the variable region. The probe for *Igk* (Vk 10) was obtained from M. SHAPIRO, ICR (Philadelphia). The clone was isolated from the PC3386 plasmacytoma nonproductively rearranged kappa gene (KELLEY *et al.* 1985). The 900 bp *Eco*RI-*Hin*dIII subclone contains the 5′ flanking region, leader peptide coding region, and the Vk coding region to within two amino acids of the Jk region.

RESULTS

Allelic composition of *Hox-1.5* among inbred and wild mice: To characterize *Hox-1.5* as a genetic marker, genomic DNAs from inbred and wild mice were examined by Southern analysis for polymorphisms in restriction endonuclease fragments (REF) hybridizing to the 1.4 kb *Eco*RI insert (Mo-10) of pMo-10. Genomic DNAs from inbred and wild mice digested with *Eco*RI were relatively non-polymorphic in their hybridization with *Hox-1.5*; only *Mus cervicolor popaeus* displayed a novel fragment. BALB/cAnPt, BALB/cHeA, STS/A and AKR/N DNAs were restricted with a panel of 9 enzymes (*Eco*RI, *Hin*dIII, *Pst*I, *Kpn*I, *Stu*I, *Msp*I, *Eco*RV and *Nco*I); *Eco*RV fragments were the only DNA fragments found to be polymorphic upon hybridization with *Hox-1.5* among these 4 strains. *Eco*RV digestion of DNA from a large panel of inbred and wild mice (Table 1) revealed two predominant haplotypes. Most mice contained two non-polymorphic *Eco*RV REF bands of 6.3 kb and 5.4 kb. However, a third polymorphic *Eco*RV REF band of 12.5 kb was characteristic of the *Hox-1.5*^{a} allele and a 16.4 kb REF band segregated as the *Hox-1.5*^{b} allele. The wild mouse species, *Mus minutoides* was unique in containing a 22-kb *Eco*RV REF band, designated *Hox-1.5*^{c}, in addition to the 5.4- and 6.3-kb nonpolymorphic bands. Although *Hox-1.5*^{a} and *Hox-1.5*^{b} are distributed with approximately equal frequency among inbred mice, the *Hox-1.5*^{b} haplotype appears to be much more common among wild mice either caught directly or recently derived from the wild (POTTER 1986).

Among the inbred mouse strains tested were several parental lines of recombinant inbred strains commonly used in chromosomal mapping studies. 020 and AKR were the only parental strains for which restriction fragment length polymorphisms for *Tcrb*, *Igk* and *Hox-1.5* were found. Segregation of the *Hox-1.5* *Eco*RV REF among the OXA recombinant inbred strains positions *Hox-1.5* to within 4.5 cM (95% confidence limits, 0.46–29.1 cM) of either *Igk* (12 of 14 concordances) or *Tcrb* (also 12 of 14 concordances) (Table 2). The strain distribution patterns among the recombinant inbreds and the presence of the *Hox-1.5*^{a}

TABLE 1

Allelic composition of *Hox-1.5* (Mo-10) sequences hybridizing to *Eco*RV fragments of inbred, recombinant inbred and wild mouse DNA

	Inbred strains	Recombinant inbred strains	Commensal and noncommensal wild mice
Hox-1.5^a: (12.5 kb)	A/J, ABP/Le, BALB/cAnPt, BALB/cByJ, BALB/cHeA, BUB/BnJ, CBA/N, CE/J, C3H/HeN, C3H/HeJ, DBA/2J, MRL/MpJ-*lpr*, NAK, NZB/BlNJ, NZW/N, 020/A, SJL/JLwPt, SWR/J	CXB: G,I,J,K CXS: A,C,E,F,K,L OXA: B,D,G,J AXB: 3,4,6,7,10,12,17,23,24 BXA: 1,4,7,8,9,10,11,12, 13,16,18,22,23,24	*M. pahari, M. caroli, M. musculus domesticus* (MD, USA populations: Haven's Farm, J. J. Down's Farm, Centreville; DE, USA population: Lewes)
Hox-1.5^b: (16.4 kb)	AKR/N, C57BL/6ByJ, C57BL/6J, C57BL/10N, C57L/J, C58/J, I/LnJ, P/J, PL/J, STS/A, YBR/Ei	CXB: D,E,H CXS: B,D,G,H,I,J,M,N OXA: A,C,E,F,H,I,K,L,M,N AXB: 1,2,8,9,11,13,14,15,18,19, 20,21,22,24 BXA: 2,6,14,19,20,25	*M. saxicola, M. shortridgei, M. abbotti, M. booduga, M. cervicolor popaeus, M. cookii, M. hortulanus, M. spretus*; *M. musculus* subspecies: *musculus* Czech I and II, *castaneus, molossinus, brevirostris, praetextus*; *poschiavinus*; *M. m. domesticus* populations: (MD, USA: Sanner's Farm, U. of MD Tobacco Farm, Centreville; CA, USA: Bouquet Canyon; Scotland: Eday, Papa Westray, Caithness, Isle of May
Hox-1.5^c: (22 kb)			*M. minutoides*

TABLE 2

Strain distribution patterns of chromosome *6* loci among the 14 OXA recombinant inbred strains

	OXA Recombinant Inbred Strains													
Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Tcrb	A	O	O	O	A	A	O	O	A	O	A	A	A	A
			×					×						
Hox-1.5	A	O	A	O	A	A	O	A	A	O	A	A	A	A
			×	×										
Igk	A	O	O	A	A	A	O	A	A	O	A	A	A	A

O and *A* are symbols for alleles inherited from the 020/A and AKR/FuRdA strains, respectively. A crossover between two loci is indicated by an ×.

allele from the NZB parent in the *Igk* recombinant mouse, NAK, support the previous location of *Hox-1.5* with respect to *Igk* on mouse chromosome *6* (McGinnis *et al.* 1984b; Bucan *et al.* 1986).

Allelic relationship of *Hox-1.5* and *Hd*: In order to assess the allelic relationship of *Hox-1.5* and *Hd*, *Hd*/+ backcross mice were examined for recombination events between this morphological marker and the *Eco*RV REF hybridizing to the homeo box sequence Mo-10. C.B6.C3-*Hd*/+ mice, tested *Hox-1.5*^{a}/*Hox-1.5*^{b} (D'Hoostelaere, Jouvin-Marche and Huppi 1985) and were mated with SJL/JLwPt (*Hox-1.5*^{a}/*Hox-1.5*^{a}) mice. The *Hd*/+ F_1 male progeny were backcrossed to SJL/JLwPt. The 88 *Hd*/+ N_2 mice resulting from this cross were tested for *Tcrb* (constant region) and *Igk* (Vk21 and constant region) (D'Hoostelaere, Jouvin-Marche and Huppi 1985). The seven mice inheriting recombinations between *Hd* and *Tcrb* (3 of 88) or *Igk* (4 of 88) (D'Hoostelaere, Jouvin-Marche and Huppi 1985), plus the remaining 77 progeny tested, showed no recombinations between *Hd* and *Hox-1.5* (0 of 84), indicating close linkage (Fig. 1).

In a separate backcross experiment, C58.B6.C3-*Hd*/+ mice (*Hox-1.5*^{b}/*Hox-1.5*^{b}) were mated with ABP/Le *wa-1*/*wa-1* mice and the *Hd*/+ F_1 male mice were backcrossed to the ABP/Le parental. The recessive marker, waved-1 (*wa-1*), is reported to be approximately 11 cM distal to *Hd* on chromosome *6* (Hummel 1970). Recombinants between *Hd* and *wa-1* were selected based on the presence or absence of both markers in the same mouse (Figure 1). Of the 404 progeny produced, 186 were *Hd*/+, +/*wa-1* and another 183 were +/+, *wa-1*/*wa-1*; these mice were not tested for *Hox-1.5*. The remaining 35 progeny inherited a recombinant chromosome between *Hd* and *wa-1*, indicating a map distance of 8.7 ± 1.4 cM between these two loci. One of the 35 *Hd* to *wa-1* recombinants died prior to further genetic testing. Of the remaining 34 mice, 15 were *Hox-1.5*^{a}/*Hox-1.5*^{b} and 18 were *Hox-1.5*^{a}/*Hox-1.5*^{a}. Only one of the 34 mice inherited a recombination between *Hd* and *Hox-1.5*. An *Hd*/+ mouse (#5949), expected to be *Hox-1.5*^{a}/*Hox-1.5*^{b}, carried the *Hox-1.5*^{a} allele (Figure 2). Of the 492 (88 + 404) N2 progeny generated during the course of the two backcross experiments, 118 (84 + 34) were typed for *Hox-1.5* alleles. Only 1 mouse inherited a

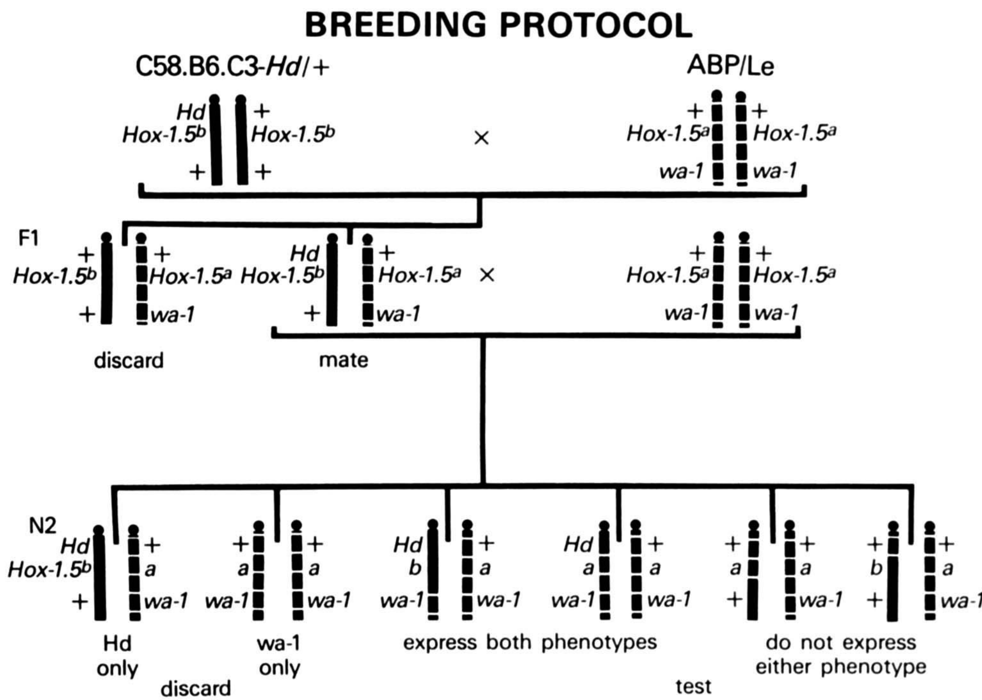

FIGURE 1. The relationship of *Hox-1.5* to *Hd* was examined by mating *Hd/+* mice (C58.B6.C3-*Hd/+*) to ABP/Le mice, another mutant stock homozygous for the morphological marker, *wa-1*. *Hd/+* F_1 mice were backcrossed to the ABP-Le parental. The resultant N_2 progeny, with the exception of mice expressing *Hd* only or *wa-1* only, were examined for cosegregation of *Hd* and *Hox-1.5* (see MATERIALS AND METHODS).

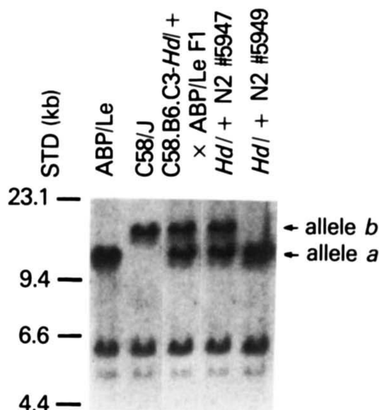

FIGURE 2.—Genomic DNA was digested with *Eco*RV, size-fractionated on agarose gels, transferred to nitrocellulose filters, and hybridized with the 1.4-kb *Eco*RI insert of pMo-10. Numbered lanes contained DNA from *Hd/+* mice detected in first generation backcrosses. N_2 mouse #5949 shows a recombination between *Hd* and *Hox-1.5*.

recombinant chromosome between *Hd* and *Hox-1.5* indicating a map distance of 0.85 ± 0.85 cM between the two loci.

DISCUSSION

The finding of allelic variation at the *Hox-1.5* locus identified this sequence as a useful genetic marker for the further characterization of the *Tcrb* or *Igk* region of mouse chromosome *6*. The gene order in this region was determined as *Tcrb-Hd-Igk* (D'HOOSTELAERE, JOUVIN-MARCHE and HUPPI 1985) based on recombination frequencies among these loci in a set of backcross mice generated from SJL and C.B6.C3-*Hd/+* parents. When these same backcross mice were typed for *Hox-1.5* alleles, no recombinations were observed between *Hd* and *Hox-1.5*. In a subsequent backcross experiment, one mouse inherited a chromosome with a recombination between *Hd* and *Hox-1.5*, thus these loci are either non-allelic or the recombination was intragenic. In addition, since *Hox-1.5* resides within a cluster of homeo box sequences, the observed recombination could also have occurred at another site within the *Hox-1* cluster.

The close proximity of a homeo box sequence, found to be expressed during mammalian embryogenesis, with the developmental mutation, hypodactyly is striking. As *Hox-1.5* represents a small portion of the *Hox-1* complex and the recombination frequency is low, it may be possible to find the actual recombination site in the mouse which inherited a recombination between *Hd* and *Hox-1.5* by using nearby flanking region probes. This study would determine the direction for chromosome walking experiments designed to aid in the cloning of the gene(s) responsible for the hypodactyly mutation. In the homozygous state, hypodactyly is generally lethal. *Hd/Hd* mice that survive are infertile and show severe abnormalities in skeletal formation. Morphological effects of the *Hd* mutation are detected during embryogenesis; in experimental studies, most of the homozygous and some of the heterozygous 16–18-day fetuses taken from uteri of *Hd/+* females, pregnant by *Hd/+* males, were dead (HUMMEL 1970). In the mouse, segmentation processes occur early in embryogenesis between the 7th and 8th days of development (HOGAN, HOLLAND and SCHOFIELD 1985; SNELL 1941). Studies of a closely linked homeo box sequence in the *Hox-1* cluster, *Hox-*

1-3 (DUBOULE *et al.* 1986), reveal that two homeo box containing transcripts are found in mouse embryos at day 9 and that only one of them is found in subsequent days up until day 16 when it also becomes undetectable.

Given the tight linkage of *Hox-1.5* with *Hd* and the fact that *in situ* hybridization can detect messages in tissue sections, it would be of interest to examine sequences within the *Hox-1* cluster for expression during the development of embryos homozygous for *Hd*. Experiments to test for differences in timing or segmental specification of expression of *Hox-1* and flanking region sequences in +/+, *Hd*/+, and *Hd*/*Hd* mouse embryonic tissue sections may be important in the pursuit of a model system for studying the regulation of mammalian development. The spatial expression and extensive interspecific sequence homology of homeo box genes suggest that mutation within these sequences is potentially deleterious.

Human cases of hypodactyly are rare due to their lethality (CHICARILLI and POLAYES 1985) and as such genetic linkage studies have not been done. Recent studies (BUCAN *et al.* 1986; RABIN *et al.* 1986) have shown that homeo box sequences within the *Hox-1* cluster are located on human chromosome 7. Several human skeletal disorders have been assigned to this chromosome (MCKUSICK 1986) and thereby become potential candidates for the examination of associations of developmental disorders with human homeo box sequences.

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