The *Hox-2* Homeo Box Gene Complex on Mouse Chromosome *11* Is Closely Linked to *Re*

Charles P. Hart,* Dyana K. Dalton,† Lois Nichols,* Lisa Hunihan,* Thomas H. Roderick,‡ Stephen H. Langley,‡ Benjamin A. Taylor‡ and Frank H. Ruddle*,†

*Department of *Biology and †Human Genetics, Yale University, New Haven, Connecticut 06511 and ‡The Jackson Laboratory, Bar Harbor, Maine 04609*

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ABSTRACT

Restriction fragment length polymorphisms have been identified between inbred strains of mice for the homeo box gene complex *Hox-2*. These genetic markers were used to follow the segregation of different *Hox-2* alleles among recombinant inbred strains of mice and among the progeny of a three point genetic cross. The results place the *Hoax-2* locus approximately 1 cM from the rex (*Re*) locus on mouse chromosome *11*.

THE homeo box was originally described as a conserved 180-bp protein coding domain present in genes of Drosophila important for regional specification within the embryo (McGINNIS *et al.*, 1984a; SCOTT and WEINER 1984). The homeo box has subsequently been shown to be present in a number of Drosophila genes and expressed in a temporally and spatially restricted manner consistent with a developmental role. The homeo box has structural features reminiscent of prokaryotic and eukaryotic DNA binding proteins (SHEPHERD *et al.* 1984; LAUGHON and SCOTT 1984) and more recently has been shown experimentally to encode a sequence specific DNA binding protein domain (DESPLAN, THEIS and O'FARRELL 1985; FAINSOD *et al.* 1986). Thus the homeo box may function through the control of batteries of other genes via specific DNA binding. Proposed models include both an auto- and trans-regulatory role for homeo box gene products (DESPLAN, THEIS and O'FARRELL 1985; HARDING *et al.* 1986; FAINSOD *et al.* 1986).

Homeo box containing genes have also been cloned from the genomes of mice, humans, frogs, and sea urchins. We have described three homeo box loci of the Antennapedia-type in the mouse genome, *Hox-1*, *-2*, and *-3*, which map to chromosomes *6*, *11*, and *15*, respectively (McGINNIS *et al.*, 1984b; RABIN *et al.* 1985; AWGULEWITSCH *et al.* 1986). By *in situ* hybridization *Hox-1* and *Hox-2* have been regionally mapped to bands B3 to C on chromosome *6* (BUCAN *et al.* 1986) and bands C to E of chromosome *11* (MÜNKE *et al.* 1986), respectively. We and others have demonstrated that two of these loci (*Hox-1* and *Hox-2*) are gene clusters containing multiple homeo box sequences, while one of the loci (*Hox-3*) appears to contain a single homeo box (HART *et al.* 1985; COLBERG-POLEY *et al.* 1985a; RUDDLE *et al.* 1985; AWGULEWITSCH *et al.* 1986, BRIER *et al.* 1986; DUBOULE *et al.* 1986). The murine homeo box loci are expressed during embryogenesis (HART *et al.* 1985; JACKSON, SCHOFIELD and HOGAN 1985; HAUSER *et al.* 1985; COLBERG-POLEY *et al.* 1985b) and regulated in a tissue-specific and stage-specific manner (AWGULEWITSCH *et al.* 1986; WOLGEMUTH *et al.* 1986; RUBIN *et al.* 1986; DUBOULE *et al.* 1986; UTSET *et al.* 1987). Homeo box loci of the engrailed-type (FJOSE, MCGINNIS and GEHRING 1985; POOLE *et al.* 1985) have also been identified in the mouse genome (JOYNER *et al.* 1985b). Human genes corresponding to the *Hox-1*, *-2*, and *-3* loci (LEVINE, RUBIN and TJIAN 1984; RABIN *et al.* 1985; JOYNER *et al.* 1985a; HAUSER *et al.* 1985; BONCINELLI *et al.* 1985; SIMEONE *et al.* 1986; RABIN *et al.* 1986; BUCAN *et al.* 1986) and the engrailed loci (G. R. MARTIN unpublished results) have also been described.

Here we describe the genetic mapping of the *Hox-2* murine homeo box cluster. We have identified restriction fragment length polymorphisms for this locus, and have used them to analyse segregation in recombinant inbred strains of mice as well as the progeny of genetic test crosses. Our results place the *Hox-2* locus proximal to the visible mutant rex (*Re*) on mouse chromosome *11*—approximately 1 cM away. This result rules out allelism of *Hox-2* with a number of loci present on mouse chromosome *11* known to be important for the proper development of the mouse. The placement of *Hox-2* on the genetic map of *Mus musculus* will facilitate the detailed analysis of this locus and should contribute to the eventual understanding of its function and evolution.

MATERIALS AND METHODS

Cloned DNA: Plasmids pMo-1.1, which contains the murine *Hox-2.3* homeo box, and pMo-4.6, which contains the *Hox-2.2* homeo box, have been previously described (HART *et al.* 1985).

Mouse genomic DNAs: Genomic DNAs were either obtained from the DNA resource of the Jackson Laboratory, Bar Harbor, Maine (inbred strains and recombinant inbred strains) or isolated from spleens and kidneys according to the procedure of GORDON *et al.* (1980) (genetic cross progeny).

DNA blot hybridizations: Approximately 10 μg of murine genomic DNA were digested with restriction endonuclease (NEB, BRL or IBI), electrophoresed in 0.8% agarose Tris-borate gels, and transferred to either nitrocellulose or Nytran (S&S) according to the procedure of SOUTHERN (1975). The filters were prehybridized for 3–12 hr at 65° in 6× SSC, 0.1% SDS, 10× Denhardt's solution, and 500 μg/ml denatured sonicated salmon sperm DNA. The filters were hybridized in the same solution with the addition of nick-translated DNA probe ($\sim 10^8$ cpm/μg) and 10% dextran sulfate. After 16–24 hr of hybridization the filters were washed at a final stringency of 0.1× SSC, 0.1% SDS at 65–68°.

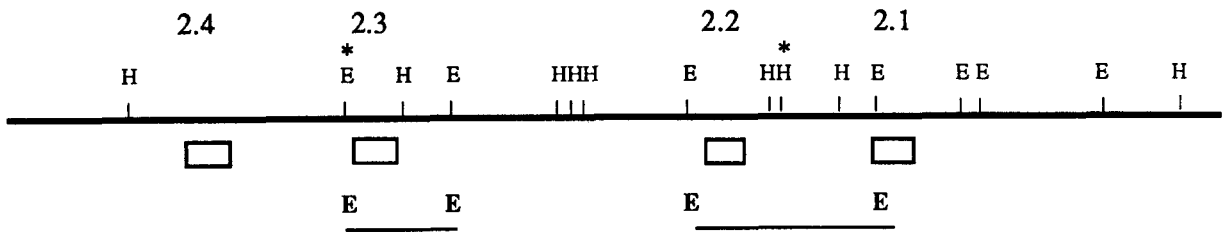
RESULTS

***Hox-2* RFLPs:** Restriction fragment length polymorphisms between different strains of mice were found at two regions of the *Hox-2* gene complex. Two alleles were identified at the *Hox-2.3* region. *Hox-2.3*^{a} is characterized by a 3-kb *Eco*RI fragment homologous to pMo-1.1. *Hox-2.3*^{b} is characterized by a 15-kb *Eco*RI fragment homologous to the same probe. The distribution of these alleles among different strains of mice is shown in Figure 1. Restriction site analysis of the two alleles shows that the 15-kb allele was the result of a deletion of approximately 1 kb of mouse genomic DNA between the *Hox-2.3* and *Hox 2.4* homeo boxes (data not shown). Alternatively, approximately 1-kb of DNA has been inserted into the *Hox 2.3*^{a} chromosome.

Three alleles have been identified in the region of the *Hox-2.2* homeo box. These are characterized by *Hin*dIII fragment differences when mouse genomic DNA is hybridized with pMo-4.6 (see Figure 2). The molecular basis for this polymorphism between *Hox-2.2*^{j} and -2.2^k appears to be a small rearrangement resulting in the loss (or gain) of a *Hin*dIII restriction site. *Hox-2.2*^{j} has hybridizing fragments of 1.9 and 0.5 kb, while *Hox-2.2*^{k} has a single hybridizing fragment of 2.4 kb. The polymorphic *Hin*dIII site is noted in Figure 1. The fact that this polymorphism is also detected with the restriction enzymes *Ava*I, *Bst*NT, *Hin*fI, and *Sph*I argues against a point mutation of the *Hin*dIII recognition sequence. The molecular basis for the *Hox-2.2*^{l} allele was not determined. The strain distribution of these restriction fragment length polymorphisms (RFLPs) is also shown in Figure 1.

Recombinant inbred analysis: The segregation of the *Hox-2.3* alleles was studied in two sets of recombinant inbred strains. Twenty-five strains of the AKXD set (AKR/J and DBA/2J progenitor strains) and eighteen strains of the AKXL set (AKR/J and C57L/J progenitor strains) were examined for their inheritence of one or both of the progenitor strain alleles. The results are shown in Table 1. The strain distribution patterns were then compared with those for other chromosome *11* markers. The AKXD set has been analysed for the *Sparc* gene [*S*ecreted *p*rotein, *a*cidic, *r*ich in *c*ysteines] (MASON *et al.* 1986) and *Akv-4* [endogenous provirus found in AKR/J mice] (JENKINS *et al.* 1981), while the AKXL set has been analyzed for *Erbb* [cellular homolog of the avian erythroblastosis virus] (SILVER *et al.* 1985), *Hba* [alpha globin] (SILVER *et al.* 1985), *Akv-4* (STEFFEN, TAYLOR and WEINBERG 1982; TAYLOR *et al.* 1985), *Glk* [galactokinase] (MISHKIN, TAYLOR and MELLMAN 1976), and *Es-3* [esterase-3] (MISHKIN, TAYLOR and MELLMAN 1976; TAYLOR *et al.* 1985). Although the strain distribution pattern of the *Hox-2* locus did not indicate tight linkage with any of the marker loci (TAYLOR 1981; SILVER and BUCKLER 1986), estimated recombination frequencies (r) were calculated by the formula $r = R/4 - 6R$, where R is the fraction recombinant inbred strains sampled that are recombinant between the alleles of interest (TAYLOR 1981). The combined estimated recombination frequency between *Hox-2* and *Akv-4* in the AKXL and AKXD sets was 0.18 (15 recombinant strains out of 43 total); the estimated recombination frequency between *Hox-2* and the *Hba* with the AKXL set was 0.27 (7 recombinants out of 17 total) and the estimated recombination frequency between *Hox-2* and *Es-3* using the AKXL set was 0.17 (6 recombinants out of 18 total). Taken together, these results suggested that the *Hox-2* locus is between *Akv-4* and *Es-3* on mouse chromosome *11*.

Genetic test crosses: Three point and two point test crosses were set up using the dominant markers trembler-J (*Tr*^{J}), a neurological mutation, and rex (*Re*), a coat mutation, along with the co-dominant *Hox-2* RFLP alleles. When the genomic DNA from a C57BL/6Ei-*Re Tr*^{J} mouse was examined for its *Hox-2.3* allele it was found to be heterozygous for the 3-kb and 15-kb *Eco*RI bands. This surprising result is probably due to the fact that the *Re* mutation placed on the C57BL/6 background was derived from an RSV/Le mouse. RSV/Le contains the *Hox-2.3*^{b} allele. Thus the development of the C57BL/6Ei-*Re Tr*^{J} line apparently led to the coselection of the linked *Hox-2* allele. This enabled us to cross C57BL/6Ei-*Re Tr*^{J} female mice heterozygous for *Hox-2.3* with C57BL/6J males which were homozygous for *Hox-2.3*^{a}. This cross is diagrammed in Figure 3. Seventy-three off-



ALLELES	*Hox-2.3*^{a}	*Hox-2.3*^{b}	*Hox-2.2*^{j}	*Hox-2.2*^{k}	*Hox-2.2*^{l}
RFLPs	3 kb	15 kb	9 kb 5.5 kb 1.9 kb 0.5 kb	9 kb 5.5 kb 2.4 kb	8 kb 5.5 kb 1.9 kb 0.5 kb
	Eco RI	*Eco* RI	*Hin*dIII	*Hin*dIII	*Hin*dIII
STRAIN DISTRIBUTION	BALB/cJ C3H/HeJ C57BL/6J C57L/J DBA/2J NZB/BINJ SWR/J	A/J AKR/J RSV/Le *M. spretus*	BALB/cJ C3H/HeJ C57BL/6J C57L/J DBA/1J DBA/2J LP/J NZB/BINJ PL/J SJL/J SM/J SWR/J 129/SvJ	A/J AKR/J CBA/J RSV/Le	*M. spretus*

FIGURE 1.—*Hox-2* alleles and their inbred strain distributions. At the *top* is a restriction map of the *Hox-2* homeo box region showing the location of *Eco*RI and *Hin*dIII restriction endonuclease cleavage sites. The boxes represent the locations of the homeo box sequences whose names are listed above. The hybridization probes are shown below. The *asterisks* indicate the sites of the regions polymorphic among the different strains of mice. Names, hybridizing fragments, and strain distributions of the *Hox-2* alleles are shown below.

spring were scored for Tr^{J}, *Re*, and their *Hox-2* alleles. A two point cross between *Re* and *Hox-2* was also used. Nineteen offspring were scored from this cross. The results from these crosses are summarized in Table 2.

The 22% recombination found between Tr^{J} and *Re* (0.219 ± 0.048) is consistent with the 18.5 cM which separate Tr^{J} and *Re* (FLANAGAN 1966). We also found an approximate 21% recombination frequency between *Tr* and *Hox-2* (0.206 ± 0.047).

Only one recombinant was found between *Re* and *Hox-2*. This allows *Hox-2* to be placed approximately 1 cM (0.0109 ± 0.0108) from the *Re* locus on mouse chromosome *11* (95% confidence limits: 0.3 and 5.8 cM). The genotype of this recombinant mouse was wild type for *Re* and heterozygous for *Hox-2*. Its trembler genotype was also heterozygous (positive for the dominant trembler phenotype)—thus the *Hox-2* locus can be provisionally placed on the trembler (centomeric) side of the *Re* locus. Maximum

TABLE 1

Recombinant inbred strain analysis of the *Hox-2* alleles

	AKXL 9AKR/J × C57L/J																		
	5	6	7	8	9	12	13	14	16	17	19	21	24	25	28	29	37	38	References
Erb b	A	L	L	L	A	L	L	A	A	A	L	A	L	A	A	A	L	A	Silver *et al.* (1985)
							×					×			×		×		
Hba	A	L	—	L	A	L	A	A	A	A	L	L	L	A	L	L	L	A	Silver *et al.* (1985)
		×		×	×				×		×		×	×			×		
Akv-4	A	A	A	A	L	L	A	A	L	A	A	L	A	L	L	L	A	A	Taylor *et al.* (1985)
							×		×		×		×			×			
Hox-2	A	A	A	A	L	L	L	A	A	A	L	L	L	L	L	A	A	A	This work
		×	×					×	×		×					×			
Es-3	A	L	L	A	L	L	L	L	L	A	A	L	L	L	L	L	A	A	Taylor *et al.* (1985)
																		×	
Glk	—	L	—	A	—	L	A	L	L	A	A	L	L	L	L	L	A	L	Mishkin, Taylor and Mellman (1976)

	AKXD (AKR/J × DBA/2J)																								
	1	2	3	6	7	8	9	10	11	12	13	14	15	16	17	18	20	21	22	23	24	25	26	27	28	References
Sparc	D	D	D	A	A	A	A	—	—	A	D	A	D	—	—	A	D	D	—	A	—	D	—	—	—	Mason *et al.* (1986)
	×	×				×					×															
Akv-4	A	A	D	A	A	D	A	D	D	A	A	A	D	D	D	A	D	D	D	A	A	D	A	A	D	Jenkins *et al.* (1981)
	×									×				×		×	×				×	×	×	×		
Hox-2	D	A	D	A	A	D	A	D	D	D	D	A	D	A	D	D	A	D	D	A	D	A	D	D	D	This work

The names and progenitor strains of the two RI sets are shown and the names of each individual strain are shown at the top of each column. The parental type of allele found for different chromosome *11* markers for each of the strains is listed in the rows. For scoring the *Hox-2* strain distribution pattern genomic DNAs were digested with *Eco*RI and hybridized with the 3-kb *Hox-2.3* homeo box hybridization probe. The ×'s denote where crossovers occurred.

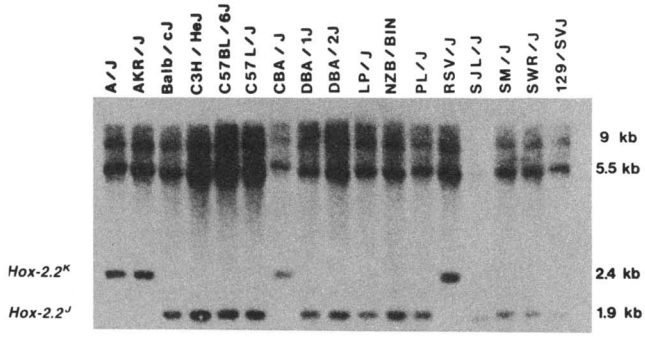


FIGURE 2.—*Hox-2.2* RFLPs. Southern blot hybridization of DNAs from various inbred strains of mice digested with the restriction endonuclease *Hin*dIII. The probe used was a 5.5-kb *Eco*RI fragment containing the *Hox-2.2* homeo box (see Figure 1). The *Hox-2.2^j* allele is characterized by four fragments of molecular weights 9, 5.5, 1.9, and 0.5 kb. The *Hox-2.2^k* allele is characterized by three fragments of molecular weights 9, 5.5 and 2.4 kb. The 0.5-kb band is not visible in this autoradiograph.

likelihood analysis (Bishop 1985) was used to measure the level of support for this gene order over the alternative gene order (Table 3). A modest level of support was found for the proposed gene order (LOD score of 0.57). Note that the likelihood analysis does not take interference into consideration. A double crossover event would be required to explain the recombinant animal's genotype if *Hox-2* is on the telomeric side of *Re*, whereas a single crossover event is sufficient if *Hox-2* is on the centromere side of *Re*. However because of the small number of recombinants the alternative order cannot be ruled out. These results are summarized in Figure 4.

TABLE 2

Summary of results from *Hox-2* genetic test crosses

Three-point test cross (C57BL/6Ei-*Re Tr^J* × C57BL/6J)

	Gametes from O parent			
Progeny class	*Tr*	*Hox-2.3*	*Re*	No. of progeny
1	+	A	+	32
2	*Tr*	B	*Re*	25
3	+	B	*Re*	12
4	*Tr*	A	+	3
5	+	A	*Re*	0
6	*Tr*	B	+	1
7	+	B	+	0
8	*Tr*	A	*Re*	0

Two-point test cross (C57BL6/Ei-*Re* × C57BL6/J)

	Gametes of O parent		
Progeny class	*Hox-2.3*	*Re*	No. of progeny
1	A	+	10
2	B	*Re*	9
3	A	*Re*	0
4	B	+	0

A and B refer to the *Hox-2* RFLP alleles *Hox-2.3^A* and *Hox-2.3^B*.

DISCUSSION

The genetic linkage map of the mouse is by far the most complete of any mammal (Roderick and

***Hox-2* THREE POINT CROSS**

C57BL/6Ei-*Re Tr*^{J} females X C57BL/6J males

$$\frac{Tr \quad Hox\text{-}2.3^{b} \quad Re}{+ \quad Hox\text{-}2.3^{a} \quad +} \times \frac{+ \quad Hox\text{-}2.3^{a} \quad +}{+ \quad Hox\text{-}2.3^{a} \quad +}$$

***Hox-2* TWO POINT CROSS**

C57BL/6Ei-*Re* females X C57BL/6J males

$$\frac{Hox\text{-}2.3^{b} \quad Re}{Hox\text{-}2.3^{a} \quad +} \times \frac{Hox\text{-}2.3^{a} \quad +}{Hox\text{-}2.3^{a} \quad +}$$

FIGURE 3.—Diagram of *Hox-2* genetic test crosses. Genotypes of paternal and maternal chromosomes are indicated. *Tr*—trembler; *Re*—rex.

TABLE 3

Maximum likelihood for gene order from the *Hox-2* three-point cross

Log likelihood for order		
Tr–Hox-2–Re	*Tr–Re–Hox-2*	*Re–Tr–Hox-2*
−18.4	−19.0	−32.8

Most likely gene order and recombination percentages (%):

Tr–21–*Hox-2*–1.4–*Re*

LOD score for order: 0.57

The order *Tr–Hox-2–Re* is 3.7 times more likely than the order with the next largest likelihood

The log likelihood for the three possible gene orders was calculated using numerical approximations of probabilities described by BISHOP (1985).

The LOD score for order is a measure of support for the indicated order. It is derived by comparing the log likelihood difference between the maximum likelihood order and the order with the second-highest likelihood (*Tr–Hox-2–Re* and *Tr–Re–Hox-2*, respectively).

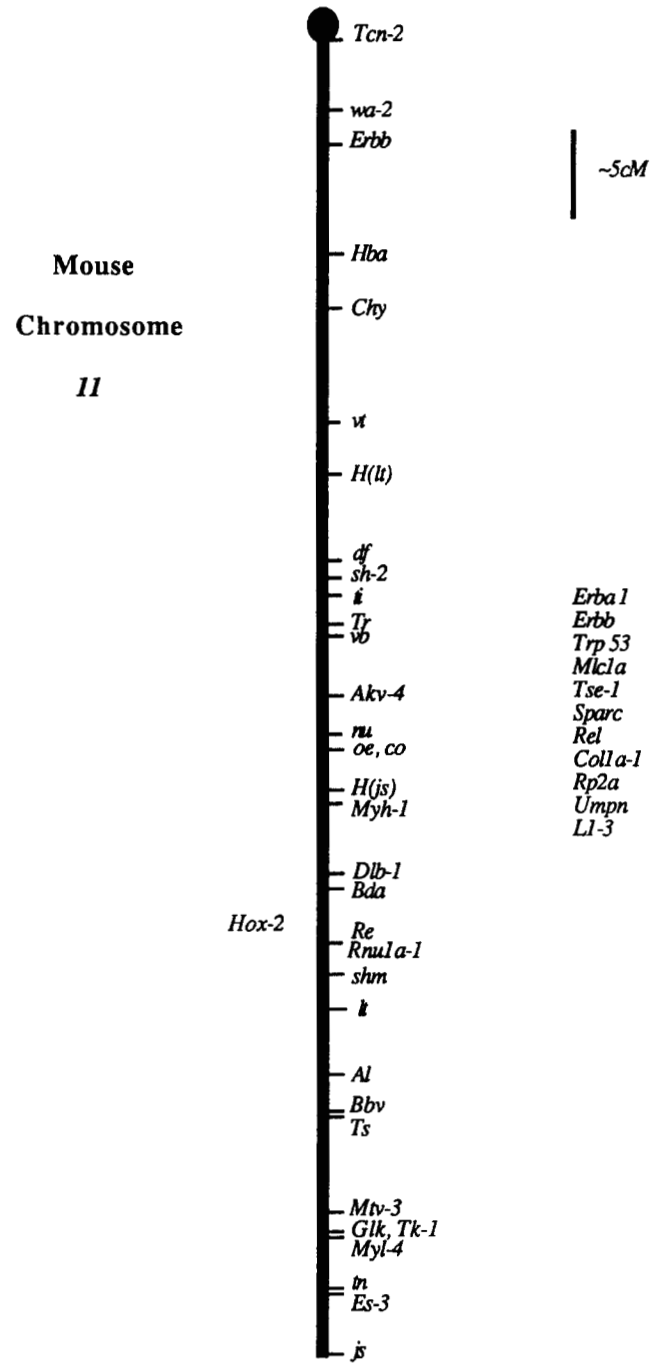


FIGURE 4.—Genetic map of mouse chromosome *11* showing provisional placement of the *Hox-2* locus 1 cM proximal of *Re*. Markers shown beneath the chromosome have been assigned to chromosome *11*, but not yet regionally localised. From DAVISSON and RODERICK (1986).

DAVISSON 1984). The development of somatic cell genetics and *in situ* hybridization has facilitated the chromosomal assignment and regional localization of genes—while the development of phenotypic marker strains and recombinant inbred strains of mice has facilitated linkage analysis and detailed recombination frequency maps. Early mouse genetic studies utilized markers affecting coat or eye color, abnormal behavioral patterns, or morphogenetic abnormalities. The identification of isoenzyme variation, and more recently, DNA restriction fragment length polymorphisms, has added numerous genes or other markers to the genetic map of Mus. Genes involved in the proper development of the mouse have been of special interest. These loci fall into two broad

classes—genes of the so-called "housekeeping" type involved in the basic structural and enzymatic processes of the cell and organism—and genes that regulate or control cell differentiation and embryonic morphogenesis. It is possible that a gene product could belong in both categories. However it is the genes of the latter type, genes whose function it is to regulate other genes, that are especially interesting in the quest for understanding developmental processes. The segmentation and homeotic genes of Drosophila are of this latter type. Mutations at these loci cause changes in cell fate—mistakes in regional specification within the embryo. The finding that many of these genes contained a similar conserved protein coding domain—the homeo box—coupled with the fact that similar sequences were present in the genomes of other insects, reptiles, amphibians, mammals, and birds—led to speculation of a common regulatory mechanism involved in early developmental decisions among diverse animal types. Questions remain as to level or extent of homology between the genes which contain the homeo box and the developmental pathways in which they are involved.

We are analyzing the organization, expression, products, and function of the homeo box genes in *M. musculus*. We hope that this endeavor will illuminate genetic and molecular processes of early mammalian development. Because of its apparent conservation we are also interested in the homeo box gene family as a paradigm for metazoan development in general. The ability to compare and contrast findings in diverse model system of development such as Drosophila, Xenopus and Mus, will contribute to the understanding of the evolution of metazoan developmental pathways.

Our previous work on the genetic organization of the homeo box gene family has included molecular studies and chromosomal and regional localizations. In this paper we have bridged part of the resolution gap between the DNA sequence and the chromosome by identifying RFLP alleles for the *Hox-2* locus and using them to examine genetic linkage with other chromosome *11* markers. We identified restriction fragment length polymorphisms at two different sites in the *Hox-2* complex. The *Hox-2.3* alleles are characterized by the presence or absence of approximately 1 kilobase of DNA between the *Hox-2.3* and *Hox-2.4* homeo boxes. The *Hox-2.2* alleles are characterized by the presence or absence of a Hind III restriction endonuclease cleavage site between the *Hox-2.2* and *Hox-2.3* homeo boxes.

We next examined the distribution of the different alleles among different inbred strains of mice. This analysis is valuable for examining the question of the independence of different alleles but in addition is useful to the study of the evolution and population dynamics of different inbred strains (MORSE 1978). The identical strain distributions that we found for the two alleles is consistent with a genetic rearrangement or rearrangements in a common progenitor mouse being responsible for the creation of both alleles—even though the molecular differences which distinguish the alleles are separated by approximately 15 kb. Detailed analysis of specific differences between representative examples of the two alleles will be necessary to explain the underlying mutations and rearrangements responsible for the differences.

We next looked at the inheritance of allelic differences among recombinant inbred strains of mice whose progenitor strains differed in their *Hox-2* alleles. Recombinant inbreds strains are generated by inbreeding the F_2 progeny produced by mating two established inbred strains (BAILEY 1971). The resulting strains are homozygous for one or the other parental type of allele. Genes that are closely linked will tend to be of the same parental type among the different strains while unlinked genes will segregate independently among the strains. The uses and limitations of recombinant inbred strains for genetic mapping and calculation of recombination frequencies between markers has been described by TAYLOR (1981) and additional considerations were recently reported by SILVER (1985) and SILVER and BUCKLER (1986). Our data using the AKXL and AKXD lines is shown in Table 1. *Hox-2* did not segregate tightly with any of the other markers for which the RI strains had been scored. Knowing that the locus was on chromosome *11* allowed us to predict its location in the vicinity of the *rex* gene. This permitted us to design a three point Mendelian test cross which is discussed below. Note that the development and utility of recombinant inbred strains is dependent on the number and location of loci for which they have been scored. Thus their utility is cumulative and increasing with the determination of the strain pattern for each new marker. Comparing the loci listed in Table 1 with their location on the genetic map of mouse chromosome *11* (Figure 4) shows that the addition of the *Hox-2* data to the data set of RI SDPs bridges the gap between *Akv-4* and *Glk* and will facilitate the mapping of new markers in this region of chromosome *11*. The results also suggest *Es-3* is proximal to *Glk* on mouse chromosome *11*. Further experiments would be necessary to determine the relative location of these two loci.

Our genetic test cross (diagrammed in Figure 3) utilized the visible markers *trembler* (*Tr*), a dominant neurological mutation which results in rapid tremors and convulsions (FALCONER 1951), and *rex* (*Re*), a dominant hair mutation resulting in wavy coats and curly whiskers (CREW and AUERBACH 1939). As discussed in the results section, the C57BL/6 derived strain, which is heterozygous for Tr^J and *Re*, is also heterozygous for *Hox-2.3*—apparently due to cose-

lecting the closely linked *Hox-2.3* allele when selecting for *Re* from the RSV/Le strain background. Results from backcrossing this dominant marker strain, or a *Re*-only marker strain, with C57BL/6J and scoring the progeny for *Tr*, *Re*, and *Hox-2* (shown in Table 2) mapped the *Hox-2* locus 1 cM from *Re*. The locus can be provisionally placed on the trembler (centromere) side of *Re* since the one recombinant between *Re* and *Hox-2* that we recovered from 73 offspring in the three point cross was heterozygous for trembler but wild type for rex. A double crossover event would be required to place *Hox-2* on the telomeric side of *Re*, whereas a single crossover event is sufficient to explain this animal's genotype if *Hox-2* is on the centromere side of *Re*. Maximum likelihood analysis (BISHOP 1985), which does not take into account interference, provided a modest level of support for this gene order over the alternative gene order (Table 3).

A recent report has demonstrated that a gene encoding a U1a1 small nuclear RNA (*Rnula-1*) implicated in the splicing of messenger RNA is also closely linked to *Re* (MICHAEL *et al.* 1986). These investigators found no recombinants between *Re* and *Rnula-1* among 22 progeny of a genetic test cross. The *Hox-2* is also closely linked to *Dlb-1*. *Dlb-1* controls the binding of a lectin from *Dolchios biflorus* to the vascular epithelium and intestinal epithelium of mice (PONDER and WILKINSON 1983). Recombinant inbred analysis and three point linkage analysis places the *Dlb-1* locus 3.1 ± 1.4 cM proximal to *Re* (UITERDIJK *et al.* 1986). The mutant *Bda* (bald-arthritic) is also closely linked to *Re* (recombination frequency of 0.032) (WALLACE and FERGUSON 1984). Homozygotes are hairless and develop a cramped gait by two months of age. It is interesting to note that both dominant hair loss and dominant hair waving mutation are located near two of the homeo box loci (*Bda* and *Re* near *Hox-2* on chromosome *11; N* (Naked) and *Ca* (Caracul) near *Hox-3* on chromosome *15*).

These findings rule out allelism of the *Hox-2* homeo box gene cluster with a number of mouse mutants located on chromosome *11* whose phenotypes indicated mutations in genes necessary for proper development. It should be noted however that mammalian loci can be quite large. The muscular dystrophy locus in humans has been estimated to possibly span over one million base pairs (KUNKEL *et al.* 1986). Thus the *Hox-2* locus may in fact be functionally linked or allelic to the closely linked markers shown in Figure 4.

We and others have previously shown on the basis of DNA hybridization, DNA sequencing, and chromosomal assignments that the murine *Hox-2* locus is homologous to a human homeo box cluster on human chromosome *17* (HART *et al.* 1985; HAUSER *et al.* 1985; RABIN *et al.* 1985; JOYNER *et al.* 1985a). The *Hox-2* loci are members of the same conserved linkage groups which include the genes for thymidine kinase, galactokinase, and the *erb-a* oncogene. Also in this region of human chromosome *17* are additional genes involved in cell growth and differentiation. These include the growth hormone gene complex, somatastatin gene complex, the *neu* proto-oncogene homolog of the epidermal growth factor receptor, the gastrin gene complex, the CD7, S9, and A12M4 surface antigens, the nerve growth factor receptor, the beta crystallin gene, carboxypeptidase A, glucosidase, and the tissue specific extinguisher locus (TSE1) (DE LA CHAPELLE 1985). We hope to make a detailed genetic map of the corresponding region of mouse chromosome *11* using interspecific cross hybridization of many of these human genes whose murine homologs may reside on mouse chromosome *11*. Whether these genes are functionally related or their relative proximity to each other is a coincidence remains to be investigated. We also plan to extend our mapping studies to the intermediate range of resolution—resolving the genetic organization between our molecular cloning of the region (1–100 kbp resolution) and our cM and cytogenetic mapping (1000–10,000 kbp resolution) using recently developed techniques of pulsed-field gel electrophoresis and long-range restriction mapping.

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