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Genetic and cytogenetic localisation of the homeo box containing genes on mouse chromosome 6 and human chromosome 7

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Probes from the m6 homeo box cluster were mapped to mouse chromosome 6 by somatic cell genetics, *in situ* hybridisation, and by a *Mus spretus*–*Mus musculus* backcross mapping system. In addition, the testis-specific homeo box containing cDNA, clone, HBT-1, has been mapped using the same backcross system and the B×D recombinant inbred strain set. Close genetic and physical linkage between the m6 cluster and HBT-1 was demonstrated, positioning these sequences to the same local cluster of homeo box containing genes. The map location of this cluster between IgK and Tcrb coincides with the morphological mutation hypodactyly (Hd). Synteny has been observed between a region of mouse chromosome 6 and the long arm of human chromosome 7 encompassing the markers Cpa, Tcrb and Try-1. Here we localise human sequences hybridising to the mouse m6 probes to the short arm of chromosome 7, breaking the region of synteny.

Key words: homeo box clustering/mouse chromosome 6/human chromosome 7/interspecies mouse backcross/hypodactyly

Introduction

Genes containing homeo box sequences have been identified in the genomes of arthropods, annelids and vertebrates (for review see McGinnis, 1985; Holland and Hogan, 1986). In *Drosophila melanogaster* clustering of homeo box containing genes has been demonstrated. In higher vertebrates, for example mouse, at least 11 homeo box containing genes, located on three different chromosomes have been shown to contain homeo box sequences homologous to a Drosophila *Antp* homeo box. The Hox-1 region maps to the proximal part of chromosome 6 (McGinnis *et al.*, 1984). The Hox-2 region has been mapped to mouse chromosome 11 (Joyner *et al.*, 1985a; Rabin *et al.*, 1985) and more recently to the distal region C→E of chromosome 11 (Münke *et al.*, 1986). Hox-3 and m31 have been mapped to chromosome 15 (Awgulewitsch *et al.*, 1986; Breier *et al.*, 1986). And, finally, one of the two regions containing homology with the *Drosophila en* gene, Mo-en1 is located on chromosome 1 (Joyner *et al.*, 1985b).

Colberg-Poley *et al.* have described three murine homeo box containing genes (m6, m5 and m2) that are clustered in 30 kb of genomic DNA (Colberg-Poley *et al.*, 1985a,b). These genes are expressed during differentiation of embryonal carcinoma (EC) cells, during embryogenesis and in different tissues of adult mice, including adult testis (Colberg-Poley *et al.*, 1985a,b). In contrast to these genes, which were first isolated as genomic clones, the homeo box containing clone HBT-1 was identified in a testis cDNA library by virtue of its homology with the Drosophila *Antp* homeo box (Wolgemuth *et al.*, 1986). Expression of HBT-1 in the adult mouse is apparently restricted to the testis, in particular to germ cells which have entered meiosis (Wolgemuth *et al.*, 1986; D.J.Wolgemuth and E.Engelmeyer, in preparation). Sequence analysis of HBT-1 showed that the homeo box contained in this clone is highly homologous to the homeo box sequences of m5 and m6, genes also expressed in adult testis.

Here we have attempted to define the chromosomal location of the m6 cluster and the HBT-1 homeo box containing gene in the mouse genome, (i) to determine if these four genes with highly homologous homeo boxes and common expression in testis are clustered in the mouse genome; and (ii) to suggest the possibility of allelism to genetically defined mutations which affect morphogenesis.

To determine the chromosomal location of the m6 region we used mouse × hamster somatic cell hybrids, *in situ* hybridization and the interspecies backcross between *Mus spretus* and *M. musculus* (Robert *et al.*, 1986). The HBT-1 clone has been mapped in the same interspecies backcross and, in addition, in the B×D recombinant inbred strain system. Following assignment of the m6 region to mouse chromosome 6, we have performed genetic linkage analysis of other gene loci previously mapped to mouse chromosome 6 (Cpa, Tyr-1, Raf-1, Tcrb and Igk).

The strong cross-hybridization of the m6 probes in the human genome has allowed us to determine the chromosomal location of a homologous human locus using somatic cell hybrids and *in situ* hybridization.

Results

Mapping of m6 cluster in mouse

The chromosomal location of the m6 region in the mouse genome was determined by Southern blot analysis of DNA isolated from 12 mouse × Chinese hamster somatic cell hybrids derived from four different strains (Francke and Taggart, 1979; Francke *et al.*, 1977) probed with the p577 fragment from the m6 homeo box region (Figure 1). These cell lines together contain the full complement of mouse chromosomes, except for chromosome 11. The 577 probe hybridized with a 3.5-kb *TaqI* fragment in the mouse genome and with a 4.5-kb fragment in Chinese hamster DNA. The pattern of mouse-specific fragment in cell hybrid DNAs was highly correlated with the presence of mouse chromosome 6 (Table I). There were multiple cases of discordant hybrids for all chromosomes other than chromosomes 1 and 6. There

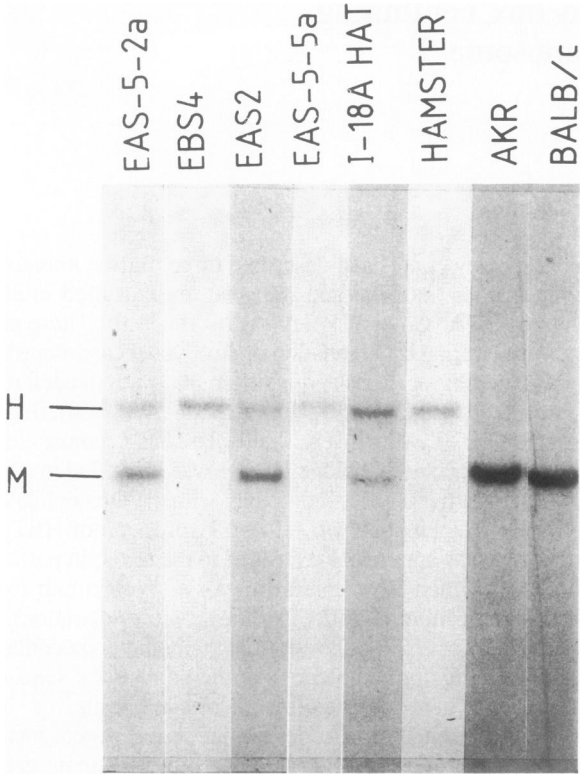

Fig. 1. Representative autoradiogram following hybridization of mouse × Chinese hamster somatic cell hybrid DNA (*Taq*I digest) with the probe 577.

was no discordancy for chromosome 6, and chromosome 1 was excluded by a single discordant hybrid.

Regional mapping of the mouse *m6* homeo box sequences was carried out by *in situ* chromosomal hybridization using probe p577. Of the cells analyzed 24.3% (10/41) had silver grains at bands B3→C of chromosome 6, 10 out of 17 grains observed on chromosome 6 (59%) were over this specific region with a peak at 6C (Figure 2). Combining our results from somatic cell analysis and *in situ* hybridization, we conclude that the mouse m6 homeo box sequences are located at bands B3→C of chromosome 6.

Mapping of m6 cluster in man

m6 homeo box sequences were assigned to human chromosome 7 by Southern blot analysis of 17 human × rodent somatic cell hybrids derived from 11 different series of hybrids (see Materials and methods). The probe from the m6 region, p577, was hybridized to *Eco*RI- or *Hin*dIII-digested hybrid cell DNAs. An 11-kb *Hin*dIII fragment or an 8.5-kb *Eco*RI fragment (Figure 3) was detected in human control DNA. A 5.4-kb *Hin*dIII fragment or a 3.9-kb EcoRI fragment (Figure 3) were observed in Chinese hamster DNA, while the mouse control had a 1.4-kb *Eco*RI band and the rat control had 4.7-kb and 3-kb *Eco*RI bands. In hybrids which contain human chromosome 7, human-specific restriction fragments, as well as rodent bands, were observed (Figure 3). The results comparing chromosome contents of this hybrid panel and human-specific bands are summarized in Table II. Chromosome 7 is the only chromosome that correlates perfectly with the m6 homeo box sequences. Every other chromosome was ruled out by at least three discordant hybrids.

Three hybrid cell lines carrying a rearranged chromosome 7 in the absence of a normal chromosome 7 were used to regionally

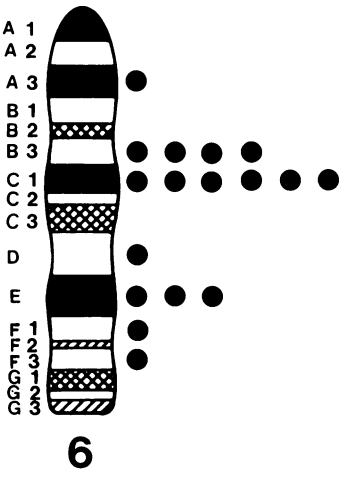

Fig. 2. Silver grain distribution along mouse chromosome 6 after *in situ* hybridization with labelled p577. The grains placed next to band C1 represent all grains over band C. [Ideogram from Nesbitt and Francke, 1973.]

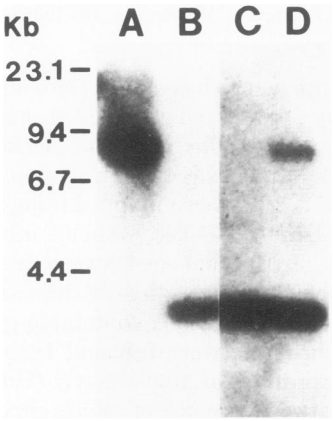

Fig. 3. Representative autoradiogram following hybridization of *Eco*RI digested DNA with p577. **Lane A**, human control DNA; **lane B**, Chinese hamster control DNA; **lane C**, hybrid lacking human chromosome 7; **lane D**, hybrid containing human chromosome 7.

localize p577 to the short arm of chromosome 7. Hybrid XVI-10C contains 7pter-7q22, XXI-23A-2c contains 7pter-7q21 and XXI-33A-b contains an isochromosome of the chromosome 7 short arm. Human m6 homeo box sequences were detected in DNA from all three hybrids, indicating that encoding human m6 homologous sequences is located on the short arm of chromosome 7. This regional assignment made by somatic cell hybrid analysis was further confirmed by *in situ* hybridization of ^{3}H-labeled p577 to normal human metaphase spreads. Fourteen out of 75 cells (18.6%) had silver grains at bands p14−p21 of chromosome 7. Of 141 total grains, 16 (11.3%) were found over this specific region with a peak at band p15 (Figure 4).

Genetic analysis using an interspecies backcross system

To correlate the chromosomal location of the m6 and the HBT-1 region, as well as gene loci previously assigned to mouse chromosome 6, we performed a genetic linkage analysis using DNA from the offspring of a backcross between *M. spretus* and *M. musculus* inbred strains (Robert *et al.*, 1985). The use of distantly related species increases the probability of finding a restriction fragment length polymorphism (RFLP) for a given

Table I. Discordancy analysis of probe 577 with mouse chromosomes in mouse × Chinese hamster hybrid clones

Hybrids	Probe 577	Mouse chromosomes 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X
I-3-2D-1d	+	+	+	−	+	−	+	+	+	+	+	−	+	L	+	+	+	+	+	+	+
I-18A HAT	+	+	+	−	+	−	+	−	−	−	+	−	+	−	−	−	+	+	L	+	+
I-18A-2a aza	−	+	+	−	L	+	L	−	−	−	+	−	+	−	−	−	−	+	+	+	−
EAS2	+	L	−	−	−	+	+	−	−	+	−	−	+	−	−	+	+	+	−	L	+
EAS5-2a	+	+	+	+	−	+	+	−	+	−	−	−	−	−	+	+	+	+	−	+	+
EAS5-4a	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	+
EAS5-4b	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	−	+	+	+	+
EAS5-5a	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−	−	−	−
EAS4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	+
EBS10	−	L	+	+	−	−	−	+	−	L	+	−	+	+	L	+	+	−	+	−	+
EBS11	−	−	−	−	−	−	−	+	−	−	+	−	+	−	−	+	+	−	−	−	+
EZS25	−	−	+	−	+	−	−	+	+	−	−	−	+	+	−	+	+	+	L	+	+
Discordant hybrids		1	4	4	3	3	0	7	3	3	6	5	6	6	3	6	6	3	4	2	5
Total hybrids		10	12	12	11	12	11	12	12	11	12	12	12	11	11	12	12	12	10	11	12

L = Data for chromosomes present at a frequency of 0.1 or less were excluded.

Table II. Discordancy analysis of probe 577 with human chromosomes in somatic cell hybrids

	Hybridization/ chromosome	Human chromosomes[a] 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
Concordant	+/+	1	2	4	2	1	2	5	4	3	1	3	3	4	6	4	3	2	3	3	2	3	5	4	1
hybrids	−/−	6	5	4	5	4	3	8	3	7	8	3	5	3	3	6	4	7	3	2	3	2	3	4	8
Discordant	+/−	6	6	4	5	6	5	0	3	5	6	4	4	4	1	4	5	6	5	5	6	3	3	2	7
hybrids	−/+	3	4	5	3	5	5	0	5	2	1	5	4	5	6	3	5	2	6	7	6	7	6	1	1
Discordant hybrids		9	10	9	8	11	10	0	8	7	7	9	8	9	7	7	10	8	11	12	12	10	9	3	8
Informative hybrids[a]		16	17	17	15	16	15	13	15	17	16	15	16	16	16	17	17	17	17	17	17	15	17	11	17
Percent discordant		56	59	53	53	69	67	0	53	41	44	60	50	56	44	41	59	47	65	71	71	67	53	27	47

[a]Data for chromosomes rearranged or present at a frequency of <1% were excluded.
In concordant hybrids the human-specific restriction fragment and the respective human chromosome were either both present (+/+) or both absent (−/−).
In discordant hybrids the human fragment was present in the absence of the respective chromosome (+/−) or vice versa (−/+).

probe, even in highly conserved regions. Here we present the linkage map of a part of mouse chromosome 6 containing the structural genes encoding T cell receptor β chain (Tcrb), immunoglobulin kappa light chain (Igk), trypsin 1 (Try-1), carboxypeptidase A (Cpa), the raf-1 oncogene (Raf-1) and homeo box containing genes from the m6 and HBT-1 region. Some of the analysed loci, such as Try-1 (Honey *et al.*, 1984b), Cpa (Honey *et al.*, 1986), and the Raf-1 oncogene (Kozak *et al.*, 1984) had been previously assigned to mouse chromosome 6, but their intrachromosomal locations were not known. Tcrb had been mapped to the proximal half of chromosome 6 by analysis of a translocation hybrid (Lee *et al.*, 1984), and by *in situ* hybridization to the band B (Caccia *et al.*, 1984). In addition to having been placed on the genetic map of chromosome 6 (Roderick and Davidson, 1983) Igk has been assigned to band 6C2, by studying different plasmacytoma-specific translocations (Wiener *et al.*, 1984; Ohno *et al.*, 1984; Webb *et al.*, 1984).

RFLPs between *M. spretus* and C57BL/6 allowed us to determine the order of several chromosome 6 specific loci using a series of backcross progeny. The segregation pattern of the m6 and HBT-1 probes and five surrounding loci in 37 backcross offspring is shown in Table IIIa. The analysis of the data is summarized in Table IIIb. The distribution pattern of *M. spretus* and *M. musculus* restriction fragments allowed us to order these loci in the linkage map. However, due to the small number of progeny analysed in this experiment, only rough estimates of distance between pairs of loci could be made.

The m6 and HBT-1 probe show the same segregation pattern of RFLPs in 37 backcross animals. Moreover, both markers hybridize to two polymorphic forms of a single large *Bam*HI fragment, suggesting their close physical linkage (Figure 5). Our data place the m6 and the HBT-1 region between the Tcrb and Igk loci (Figure 6). Try-1 and Tcrb have identical segregation patterns. In addition, we can assign the Cpa locus proximal to the Try-1 and Tcrb loci, and the Raf-1 oncogene distal to the Igk locus. Our mapping results extend the previously reported cytogenetic analysis of the regional chromosomal location of Tcrb and Igk genes.

Genetic analysis using BxD RI strains

For the more precise localization of the m6 and HBT-1 probes we investigated the possibility of mapping these probes in one of the recombinant inbred (RI) strain systems (Taylor, 1978).

Several probes from the m6 region, covering 20 kb of genomic DNA were checked for polymorphism between AKR, DBA/2 and C57BL/6 using seven enzymes. These inbred strains represent the parental strains for two sets of RI lines, AKR × DBA/2 (AKxD) and C57BL/6 × DBA/2 (BxD). No RFLP was observed

within the m6 region, suggesting very high conservation. HBT-1, however, detected polymorphism between DBA/2 and C57BL/6 in *Xba*I-digested DNA. We used this polymorphism for linkage analysis of the loci previously mapped to the proximal part of the mouse chromosome 6. We tested DNA of the 26 B×D lines and obtained the strain distribution pattern shown in Table IV. The comparison of the strain distribution pattern (SDP) of HBT- with γ-glutamyl cyclotransferase locus (Ggc) and lymphocyte alloantigen locus (Lyt-2) (Tulchin and Taylor, 1981) loci indicates that HBT-1 is linked to the Ggc locus (two recombinants out of 26 RI lines).

Discussion

Homeo box sequence containing genes in mammals have been implicated as encoding functions of importance during develop-

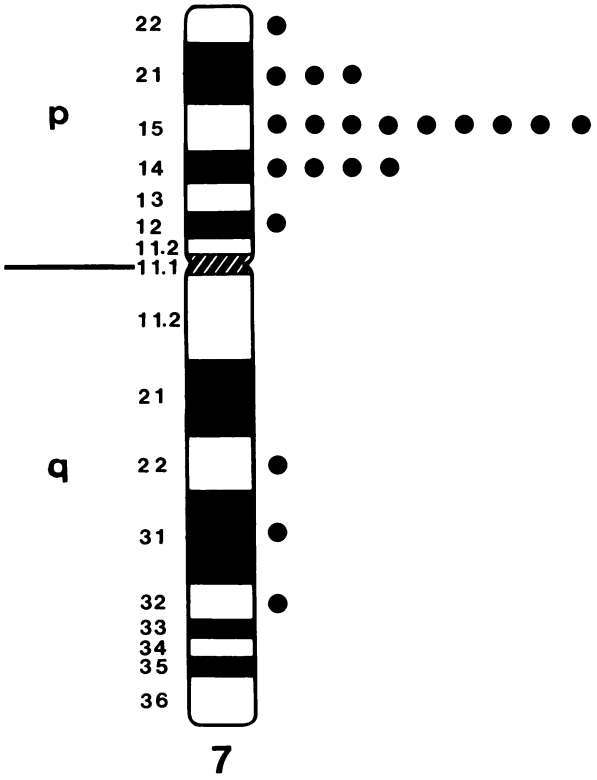

Fig. 4. Silver grain distribution along human chromosome 7 following *in situ* hybridization with p577. (Ideogram from ISCN 1985; An International Cytogentic Nomenclature, S.Karger, Basel.)

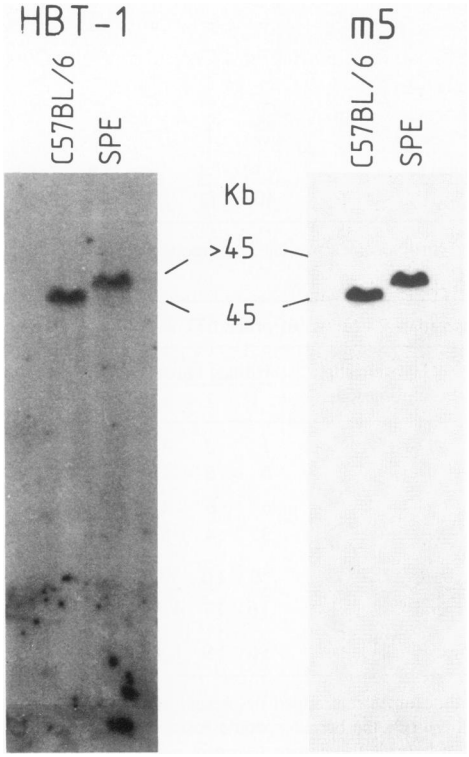

Fig. 5. Autoradiogram following hybridization of C57BL/6 and SPE DNA (*Bam*HI digest) with HBT-1 and m5 probes.

Table III(a). Strain distribution of DNA polymorphism detected by several chromosome 6 specific probes in a backcross: (B6CARB.I. × SPE)♀ × B6CARB.I.♂

Loci	Backcross progeny																																					
	1 0	1 1	1 2	1 3	1 4	1 5	1 6	1 7	1 8	1 9	2 0	2 1	2 2	2 3	2 4	2 5	2 6	2 7	2 8	2 9	3 0	3 1	3 2	3 4	3 5	3 6	3 7	3 8	3 9	4 0	4 1	4 2	4 3	4 4	4 5	4 6	4 7
Cpa	–	–	–	–	–	–	–	+	–	–	–	+	–	–	–	–	–	+	+	+	+	+	–	+	+	–	–	–	–	+	+	+	+	+	–	+	–
Tcrb Tyr-1	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	+	+	+	–	+	+	–	–	–	–	+	+	+	+	+	–	+	–
m6 HTB-1	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	+	+	+	+	+	–	–	–	–	+	+	+	+	+	–	+	–
Igk	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	–	–	–	+	+	+	+	+	+	+	–
Raf-1	+	+	–	–	–	–	–	+	–	–	–	+	+	+	–	+	+	+	–	–	+	+	+	+	+	–	+	–	–	+	+	+	+	+	+	+	–

(b). Recombination frequencies between pairs of loci:

	Igk	m6	Tcrb	Cpa
Raf-1	5/37	9/37	11/37	11/37
Igk		4/37	6/37	8/37
m6			2/37	4/37
Tcrb				2/37

(+) strands for presence of the *M. spretus* restriction fragment.

ment by analogy to the genes isolated from *D. melanogaster* (Gehring, 1985). Similar to observations in that system, a number of genes have been found to be distributed on a few mouse chromosomes. We have carried out a detailed genetic and physical mapping analysis of genes from the m6 cluster and HBT-1, a newly isolated homeo box gene showing specific expression in adult testis.

Such an investigation has two major aims. Establishing the position of potential developmental genes on the mammalian, and especially mouse, genome immediately offers the possibility of identifying previously characterised and mapped mutations as candidates for being due to lesions in the corresponding gene loci. On a smaller scale, establishment of local clustering can offer hints to function.

We have therefore used a number of genetic and physical mapping procedures to analyse the location of homeo box sequence containing genes from the m6 cluster, as well as HBT-1, a homeo box gene found to be expressed specifically in adult testis. In addition, the position of the human m6 homologue was determined by *in situ* hybridization and somatic cell genetics. Since none of the characterised human homeo box genes have yet been localised to this region, no correspondence to any published human homeo box clone can be established.

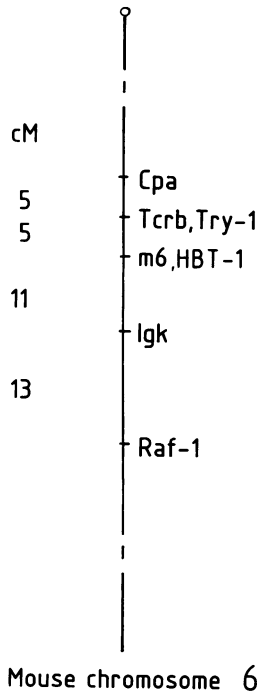

Fig. 6. Linkage map of the loci surrounding m6 – HBT-1 homeo box region.

In this study an extended genetic map of the mouse chromosome 6 was established, using a number of previously described markers. The use of multiple mapping techniques offers both an internal test for consistency of the localisation and allows the alignment of genetic and physical maps using the multipally mapped markers as milestones.

The genes from the m6 cluster are located genetically between the Tcrb and the Igk loci on mouse chromosome 6. Physical localisation by *in situ* hybridization places this locus to band B3→C of chromosome 6, in agreement with previous results positioning the flanking markers Tcrb and Igk to the bands B and C. The testis-specific homeo box gene HBT-1 shows in the backcross system the same strain distribution (zero recombinants from 36 strains) as the m6 cluster. Both markers hybridize to two polymorphic forms of a single large *Bam*HI fragment. The shift in hybridization of both markers depending on the observed polymorphism essentially rules out co-migration of independent fragments. Additional support for linkage of these genes comes from the observation of a shared *Not*I fragment of ~100 kb in pulsed field gradient gel electrophoresis experiments (D.P.Barlow and M.Bućan, unpublished data). These results would clearly define HBT-1 as a member of a local cluster of homeo box genes, including the genes for m6, m5 and m2. This clustering is of interest since the homeo box regions of these genes are highly conserved, although the non-homeo box sequences diverge completely (Colberg-Poley *et al.*, 1985a,b; Wolgemuth *et al.*, 1986). Recently, a 75-kb DNA fragment has been isolated on overlapping cosmids (Duboule *et al.*, 1986). This segment contains the m6 cluster, Mo-10 and two additional homeo box containing genes (Hox1-3 and Hox1-y). The proposed correspondence of the Hox1-3 and HBT-1 is further supported by the results reported here.

Subchromosomal mapping results for Mo-10, based on somatic cell hybrids in which the region 6B1/B2 to centromere was excluded for this gene (McGinnis *et al.*, 1984), are in contradiction with the mapping data presented here. Our results, obtained by the combined approaches of *in situ* hybridization and genetic mapping, clearly place the closely linked m6 cluster within the region excluded by previous reports.

The *M. spretus* backcross system used here has a number of advantages over the more commonly used recombinant inbred strain mapping. An enormous advantage, especially in highly conserved regions like the homeo box clusters is the very high frequency of polymorphisms observed between *M. spretus* and *M. musculus*. Furthermore, the backcross progeny reflect the recombination events that occurred during a single meiosis. It is therefore possible to observe linkage over much larger distances than in the RI strain system. On the other hand, the resolution of the RI strain mapping is significantly higher, since it reflects the effect of approximately four meiosis between heterozygous chromosome stretches taking place during inbreeding (Taylor,

Table IV. Recombinant inbred strain (BxD) segregation pattern of chromosome 6 loci

Loci	BxD																													
	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
HBT-1	B	B	–	–	B	D	–	D	B	–	D	B	D	B	B	B	–	B	D	D	B	D	B	B	D	–	D	D	B	D	B	B
Ggc[a]	B	B	–	–	B	D	–	D	B	–	D	B	D	B	B	B	–	B	D	B	D	D	B	B	D	–	D	D	B	D	B	B
Lyt-2[a]	D	B	B	D	D	D	–	D	B	–	D	B	D	B	B	B	D	B	D	B	D	D	B	D	D	–	D	B	B	B	B	B

[a]Data from Tulchin and Taylor (1981).
B, C57BL/6 alelle; D, DBA/2 allele; (–), data not shown.

1978). A potential source of difficulties in the spretus backcross system is that, in general, for each marker only the presence or absence of the spretus form will be scored. Since the F1 generation (*M. spretus*/*M. musculus*) is crossed back to *M. musculus*, the musculus form will always be represented in either one or two copies, a difference too difficult to score. Therefore the resulting map positions reflect the gene order in the *M. spretus* genome. Though differences in gene order between these species could be envisaged, no differences between *M. spretus* and *M. musculus* maps for X chromosome could be detected (P.Avner, L.Amar, D.Arnaud and J.Cambrou, in preparation).

This work provides the first regional localisation of the additional markers Raf-1, Cpa and Try-1, and has established a linkage map covering a large fraction of mouse chromosome 6. Using *Xba*I restriction fragment polymorphic between C57BL/6 and DBA/2, the HBT-1 probe could be localised relative to Ggc, a marker previously located on chromosome 6. Though the strain distribution pattern observed for this marker in itself does not allow its positioning relative to Igk (Ben Taylor, personal communication), combination of spretus backcross results with data from B×D mapping would indicate the order centromere – HBT-1 – Ggc – Igk.

In analogy to the situation found in the case of the Hox-2 cluster (Münke *et al.*, 1986), where the regional localisation on chromosome 11 suggested the Tail-short (Ts) locus as a possible allelic mutation, a developmental mutation close to the m6 cluster is presented by hypodactyly (Hd). This mutation is lethal *in utero* in most homozygotes and causes monodactyly, while in heterozygotes only the distal phalanges are absent on the first of the hindlegs (Hummel, 1970). Detailed linkage analyses have placed Hd between Tcrb and Igk (D'Hoostelaere *et al.*, 1985). The map position coincides, within the resolution of both mapping experiments, with our mapping of the m6 cluster and suggests the possibility that Hd mice carry a mutation in one of the homeo box genes of this cluster.

Comparative mapping between mouse and human has revealed the conservation of syntenic groups of genes for several chromosomes (Lalley *et al.*, 1978; Nadeau and Taylor, 1984). A group of loci on the distal long arm of human chromosome 7, comprising gene loci TCRB, CPA and TRY-1, has a syntenic homologue on mouse chromosome 6 proximal to the m6 locus (Honey *et al.*, 1984a,b; Collins *et al.*, 1984; Barker *et al.*, 1984; Caccia *et al.*, 1984). Our results give additional information about the extent of this syntenic group. The m6 region, although linked to Tcrb and Try-1 loci in the mouse genome, maps instead to the short arm of the chromosome 7. The next distal locus on mouse chromosome 6, Igk, has been assigned to the human chromosome 2 (Malcolm *et al.*, 1982). Moreover, the Raf-1 locus, which maps distal to Igk according to our results, has been assigned to human chromosome 3 (Bonner *et al.*, 1984). Our data show that the region on the mouse chromosome 6: m6–Igk–Raf-1 is not syntenic in the human genome. On the basis of accumulated data on the lengths of chromosomal segments conserved in mouse and man (Nadeau and Taylor, 1984), it is evident that the region Tcrb–m6–Igk–Raf-1 represents an evolutionarily highly rearranged chromosomal region, since the gene loci linked together on the ~25–30 cM long strand of chromatin in mouse are homologous to four different chromosome regions in human. The finding that the human homeo box locus maps to the short arm of chromosome 7 could conceivably be due to a intrachromosomal rearrangement during evolution.

Materials and methods

Mice

The *M. spretus* strain SPE was founded from a few wild breeders trapped close to Granada in Spain and inbred for over 25 generations. The *Mus m.domesticus* counterpart of this interspecies backcross was a recombinant inbred strain between CBA and C57BL/6. B×D RI strains were purchased from the Jackson Laboratory, Bar Harbor, Maine.

Cell lines

Fifteen human × Chinese hamster somatic cell hybrids of series XII, XIII, XV, XVII, XVIII, XXI, 28, 29 and 31, one human × mouse hybrid XVI-10C and one human × rat hybrid XIX-25AHAT were used for mapping m6 sequences in the human. Their derivations and chromosome contents have previously been published (Francke *et al.*, 1976; Francke and Pellegrino, 1977; George and Francke, 1977; Oliver *et al.*, 1978; Francke, 1984; Pearson *et al.*, 1983; Francke and Francke, 1981; de Martinville *et al.*, 1985). The origin of Chinese hamster × mouse somatic cell hybrid clones has been described (Francke *et al.*, 1977; Francke and Taggart, 1979).

In situ hybridization

The m6 probe, p577, was nick-translated with tritiated dATP, dCTP and dTTP to a sp. act. of 1.9×10^7 c.p.m./μg. *In situ* hybridization to human and mouse chromosome preparations was carried out as published previously (Harper and Saunders, 1981; Yang-Feng *et al.*, 1985).

Hybridization probes

The following cloned probes were used in genetic analysis of chromosome 6 specific loci:

locus m6: (i) p577 probe is 1.7-kb *Eco*RI fragment from m6 recombinant phage; (ii) m5 probe is 1.9-kb *Eco*RI fragment from m6 recombinant phage (Colberg-Poley *et al.*, 1985b)
locus HBT-1: 750-bp *Pst*I/*Hin*dIII genomic fragment located 3′ to the homeo box sequence (Wolgemuth *et al.*, 1986; R.N.Duggal, E.Engelmeyer, S.Drosinos and D.J.Wolgemuth, in preparation)
locus Igk: pT1 probe is 2.5-kb *Eco*RI/*Hin*dIII insert from the V-region of the Igk gene (Steinmetz and Zachau, 1980)
locus Raf-1: 1.2-kb *Xho*I/*Bst*EII fragment from the 3611-MSV clone (Rapp *et al.*, 1983)
locus Tcrb: 4.1 cDNA probe (Snodgrass *et al.*, 1985)
locus Try-1 rat cDNA clone 4-79 (Craik *et al.*, 1984)
locus Cpa: rat cDNA clone 11-3 (Quinto *et al.*, 1982).

RLPs between *M. spretus* and *M. musculus* were detected in *Taq*I-digested DNA for Try-1, Tcrb, Raf-1 and Igk, *Bam*HI-digested DNA for m5 and HBT-1, and *Eco*RI-digested DNA for Cpa. The HBT-1 probe detected polymorphism between C57BL/6 and DBA/2 in *Xba*I-digested DNA.

Preparation of hybridization probes

Insert DNA was purifed by electrophoresis on low melting point agarose, followed by agarase treatment. The DNA band was cut out, the agarose melted at 65°C in the presence of 100 mM NaCl and 10 mM EDTA (F.Michiels, personal communication). The sample was cooled to 37°C and 40 μl/ml of agarase (Calbiochem, GmbH) was added. The digestion was allowed to continue 2–12 h. Then the DNA was phenol extracted and traces of phenol removed by ether extraction. Finally the DNA was ethanol precipitated.

Hybridization probes were radiolabeled to a sp. act. of 3×10^9 c.p.m./μg with [^{32}P]dCTP using the Klenow fragment of DNA polymerase I and random hexamer priming (Feinberg and Vogelstein, 1983).

Southern blots

Total cellular DNA was isolated from mouse spleen by standard techniques (Blin and Stafford, 1976). Mouse genomic DNA was digested, electrophoresed and blotted onto a Gene Screen membrane by capillary transfer in denaturation solution (0.5 M NaCl and 0.5 M NaOH) as described by Herrmann *et al.* (1986). The filter was exposed to u.v. light and hybridized according to Church and Gilbert (1984) using 2×10^6 c.p.m./ml hybridization solution.

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