New Molecular Markers for the Distal End of the *t*-Complex and Their Relationships to Mutations Affecting Mouse Development

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ABSTRACT

Many mutations affecting mouse development have been mapped to the *t*-complex of mouse chromosome *17*. We have obtained 17 cosmid clones as molecular markers for this region by screening a hamster-mouse chromosome *17* and *18* cell hybrid cosmid library with mouse-specific repetitive elements and mapping positive clones via *t*-haplotype *vs.* C3H restriction fragment length polymorphism (RFLP) analysis. Twelve of the clones mapping distal to *Leh66B* in *t*-haplotypes are described here. Using standard RFLP analysis or simple sequence length polymorphism between *t*-haplotypes, exceptional partial *t*-haplotypes and nested sets of inter-*t*-haplotype recombinants, five cosmids have been mapped in or around *In(17)3* and seven in the most distal inversion *In17(4)*. More precise mapping of four of the cosmids from *In(17)4* shows that they will be useful in the molecular identification of some of the recessive lethals mapped to the *t*-complex: two cosmids map between *H-2K* and *Crya-1*, setting a distal limit in *t*-haplotypes for the position of the t^{w5} lethal, one is inseparable from the t^{w12} lethal, and one maps distal to *tf* near the $t^0(t^6)$ lethal and *cld*.

WHILE molecular genetic approaches to understanding developmental processes have met with considerable success in simpler experimental organisms, early mammalian development has long been a formidable challenge to molecular technology. In the mouse many of the interesting embryo-expressed genes which have been described in recent years were derived from conserved invertebrate sequences (*e.g.*, the *hox* clusters) (GRAHAM, PAPALOPULU and KRUMLAUF 1989; KESSEL and GRUSS 1990) or oncogenes (*e.g.*, the *int* genes) (JAKOBOVITS *et al.* 1986; ADAMSON 1987), and tended to be expressed around mid-stage or later in embryogenesis. While these remain valuable sources for mammalian genes involved in development, other strategies also hold great promise. New methods used to identify functionally interesting embryo-expressed genes, such as enhancer and promoter trapping, insertional mutagenesis (GOSSLER *et al.* 1989), and targeted mutagenesis (ZIMMER and GRUSS 1989) will no doubt provide significant contributions. Also, the cloning of the *T* gene, required for mesoderm formation (HERRMANN *et al.* 1990), and the identification of the first early embryo transcription factor, *Oct-4* (SCHÖLER *et al.* 1990), both of which map to the *t*-complex of mouse chromosome *17*, demonstrate the effectiveness of other approaches.

A well established and promising system for identifying early mouse developmental genes is the mouse *t*-complex. The *t*-complex of the mouse is a relatively well characterized region (BENNETT 1975; FRISCHAUF 1985) representing ~1% of the mouse genome. It occupies some 30–40 Mb of the proximal end of mouse chromosome *17* and has several distinguishing features. A *t*-bearing chromosome has several large inversions that make up the *t*-complex and isolates the region recombinationally from wild-type chromosomes (SHIN *et al.* 1983; HERMANN *et al.* 1986; HAMMER, SCHIEMENTI and SILVER 1989). Several mutations that map to this region affect sperm function (LYON 1984, 1986; SILVER and REMIS 1987) and over 20 mutations affect early or mid-stage embryogenesis (BENNETT 1975; BABIARZ 1983; ARTZT 1984). Molecular identification of the genes involved has been a difficult task for several reasons, one of the primary ones being the lack of sufficient DNA markers for such a large chromosomal region.

The production of a detailed and balanced genetic and physical map would be a significant aid in the molecular characterization of this region. While a large number of DNA probes have been mapped to the *t*-complex (Committee on Mouse Chromosome *17* 1991), the distribution of these probes is far from even and a physical map based on large pulse-field gel (PFG) separated fragments might necessarily remain incomplete. Experience with human chromosome *21* shows that the use of 67 probes on PFG blots was insufficient to link all of the 50 Mb q arm since many gaps remained. Sixty percent of these probes were physically linked on 11 *Not*I fragments. The *Not*I fragments identified by all probes totaled about 43 Mb (86%). The remaining gaps were thought to be around CpG dense regions which have a high density

T. E. dedicates this paper to DOROTHEA BENNETT in memory of her many achievements as an educator and investigator in mouse genetics.

of sites for the rare-cutting restriction enzymes used (GARDINER *et al.* 1990). With the number and distribution of probes currently mapped to the *t*-complex, it is doubtful that a level of coverage equivalent to human *21q* could be achieved.

In order to enhance the physical and genetic map of this region, we sought new DNA markers specifically for the *t*-complex by screening a hamster-mouse chromosome *17* and *18* cosmid library with mouse-specific repetitive elements followed by *t*-haplotype *vs.* C3H restriction fragment length polymorphism (RFLP) analysis of the positive clones. In this report we describe the results of this work. Seventeen cosmids were obtained which map to the *t*-complex. The map positions of 12 of these clones within the *t*-complex and their relationship to known developmental mutations on chromosome *17* are discussed. The remaining five cosmids, all of which map centromeric to the *Leh66B* locus in *t*-haplotypes are discussed in some detail in a companion study (EBERSOLE, RHO and ARTZT 1992).

MATERIALS AND METHODS

Mice: Mice carrying the entire *t*-complex or partial recombinants are congenic on the inbred strain C3H/DiSn (C3H) or approaching that state. The breakpoints of the partial *t*-haplotypes t^6 and t^{w18} are as described in Committee on Mouse Chromosome *17* (1991). Tt^6 is described in SILVER and ARTZT (1981), and HOWARD *et al.* (1990). The t^{w124} proximal partial *t*-haplotype was derived from C3H.t^{w75} (ARTZT, BABIARZ and BENNETT 1979; HOWARD *et al.* 1990). t^{h20} (LYON and BECHTOL 1977) was a gift from LEE SILVER, Princeton University. The inter-*t*-haplotype recombinants analyzed were generated from the parental *t*-haplotypes t^{w5}, t^{12} or t^{w32}, Tt^{s6}, and t^{w73}. Most of the recombinants had been typed for the presence of the relevant *t*-lethals. Anchor loci in the distal inversion already defined in these recombinants were *TL*, *H-2D*, *H-2K*, *Crya-1*, *Pim-1*, *tf*, and in those that were derived from t^{w73}, *cld* (ARTZT 1984). Not all the recombinants were typed for each of the anchor loci.

Nomenclature: Because all the loci described here are on chromosome *17*, an abbreviation of the formal name will be used for easier reading. Thus *D17Au126* will be called *Au126*, etc. For the same reason the full name for the *t*-lethals will be abbreviated, that is, *tcl-*t^{w5} will be called t^{w5}, etc., whereas the *t*-complex bearing chromosome with the mutation will be called the t^{w5} haplotype.

Primers and probes: Table 1 lists the primers used at each of the $(CA)_n$ blocks and other relevant information. Optimized $MgCl_2$ concentrations vary depending on trace EDTA amounts. Genomic DNA (stored in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) was diluted in dH_2O to 100 ng/μl before addition to the polymerase chain reaction (PCR).

PCRs were as follows: 100 ng genomic template DNA were added to a reaction mix of 70.0 μM dNTPs, 1.0 μM of each primer, 0.25 μCi [^{32}P]dCTP (3000 Ci/mmol, 10 μCi/μl), 0.75 unit *Taq* polymerase (Perkins-Elmer Cetus, U.S. Biochemical), 1 × buffer (Perkins-Elmer Cetus, U.S. Biochemical, no $MgCl_2$), 1.0–1.5 mM $MgCl_2$, in a 25-μl volume. This was overlaid with mineral oil and cycled as follows: 3 min, 95°, initial denaturation (*Taq* polymerase present), then 30 cycles of (1) annealing, 60 sec, (2) extension at 72°, 60 sec, (3) denaturation at 94°, 30 sec. The final extension step was done at 72° for 15 min. PCR products were separated by polyacrylamide gel electrophoresis (6% gels).

Probe P126 is a 1.4-kbp *Bam*HI genomic fragment from cosmid 126. Probe P7 is a 1.9 *Eco*RI genomic fragment from cosmid 7. Probe P64 is a 1.4-kbp *Bam*HI genomic fragment from cosmid 64.

Screening for $(CA)_n$ repeats: Cosmids from each locus were digested with the blunt-end frequent cutters *Hae*III, *Alu*I, *Rsa*I and *Hin*cII, either singly or in double digests. The digested DNA was then separated on an agarose gel, blotted and hybridized with a ^{32}P-end-labeled $(CA)_{16}$+C oligomer. DNA digests from enzymes or enzyme combinations which gave positive fragments less than 600 bp were shotgun subcloned into the *Sma*I site of plasmid vector pBluescript II KS (Stratagene) or PUC 18. Colony blots of transformants were hybridized with the labeled $(CA)_{16}$+C oligomer to identify a set of subclones with small inserts for sequencing. Primers for PCR were synthesized on an ABI model 381A.

Cell hybrid and DNA libraries: The cell hybrid R4 4.1 was originally produced by SMILEY *et al.* (1978). It contains mouse chromosome *17* and *18* (derived from C3H) in a translocation resulting in a single metacentric murine chromosome on a Chinese hamster background. The genomic library was constructed by G. GUMMERE in this laboratory by ligating an *Mbo*I partial digest into the *Bgl*II site of cosmid vector PTL5 (HOWARD *et al.* 1990).

Screening the genomic library: To avoid the secondary screening of large numbers of clones, the library was plated at a low density of 2–4000 per 150 mM plate. Thirty-six plates were screened with an average of 10–15 positive colonies per plate. Of 402 clones picked in the primary screen 70% were single colony isolates. The remainder were brought through a standard secondary purification. Verification of the mouse origin of the cosmid insert of the single colony picks from the primary screen was performed by picking them onto a filter with a grid drawn on it, regrowing the colonies and reprobing duplicate blots of the grid with Bam5R. Following secondary screening 212 clones remained. Fifty-three duplicate clones were eliminated based on identical *Bgl*II digestion patterns and mouse liver DNA probe hybridization patterns. Fourteen Chinese hamster clones which had cross-hybridized strongly with Bam5R during the original screening were identified when they hybridized strongly with R4 4.1 genomic DNA, but weakly with mouse DNA during the mapping of the clones (see RESULTS). Ultimately, 145 mouse-derived cosmid clones from the Bam5R screen were analyzed for map position in the *t*-complex. Added to this were 28 clones derived from a previous screen using the VL30 and IAP repeated elements as probes (HOWARD *et al.* 1990). For mapping to the *t*-complex, 8–10 ug of genomic DNA from C3H, C3H.t^{12}/t^{w5}, and C3H.t^{12} mouse livers were separately digested with four enzymes, *Bam*HI, *Eco*RI, *Hin*dIII and *Taq*I or *Msp*I. Each whole cosmid clone from minipreps was used as a probe after precompetition of repetitive elements with an excess of cold mouse liver DNA (see below).

Blotting and hybridizations: Genomic digests were electrophoresed in a 0.7% agarose gel in TPE buffer for 15–17 hr at ~0.7 V/cm. Transfer of DNA was done by standard capillary blotting or by vacuum on a homemade device. Transfer buffer was either 20 × SSC (Hybond-N) or 0.4 N NaOH (Hybond-N+) (Amersham). Colony blots on Hybond-N membranes were hybridized with random primed Bam5R DNA at 2–3 × 10^6 cpm/ml according to manufacturer's instructions. Hybridizations of cosmids to genomic blots were done as follows: Prehybridization of the blot was done in 50% formamide, 50 mM $NaPO_4$, pH = 6.5, 2.5 × Denhardt's solution, 5 × SSC, 0.1% sodium dodecyl sulfate (SDS), 1 mM EDTA, with 200 μg/ml sonicated salmon sperm

TABLE 1

Primers used at the (CA)$_n$ sites

Locus	Simple sequence	Primers	PCR product bp (C3H)	Length variation	Mg^{+2} mM/ annealing °C
Au7	$(CA)_6GA(CA)_{12}$	TAC TAT GTG ACT GAG GTC AG GCC AGT GTG GTC TAT AAA TC	180	BTBRTF = C3H < $t^{w5} = t^{12} < t^{w12}$	1.0/53
Au7	$(CT)_8(CA)_5TA(CA)_{18}$	ATG CTA TAA GGG GCA TTC G CTA CAC TGA GCT AGG AAG A	182	C3H = t[a]	1.5/52
Au16	$(CA)_{17}$	TAT GGT GCA CTC TCA GTA CT ATT GAG GCA GGT TGA GTG AT	130	C3H < *t*	1.5/53
Au57	$(CA)_{19}$	TAC TGC CAT TAG TGA TCC AT GAA GTA TGT TAA GGA AAA GC	105	C3H > BTBRTF > *t*	1.0/52
Au57	$(CA)_6T(CA)_{27}$	TTG ATT TCT GCA CAT ATG CC CCT CTT GGT TGC TTA GAA G	~180	BTBRTF ≥ C3H > *t*	1.0/51
Au100	$(CA)_{44}$[b]	GAT GGC TAT TGG TGT TGA AC GAG TCT AGA CAC CTA GCA AT	~200	BTBRTF > C3H > *t*	1.5/55
Au100	$(CA)_{20}CG(CA)_{10}N_{36}(CA)_{15}$	GCC TGG TCT GAA GTA AGT C CTA CTG TTC TGA GGT CCA G	213	C3H > BTBRTF > *t*	1.0/55
Au126	$(CA)_{22}$	AAG CTT GGG AAA ACA CTG CT TTG GCA GCA CCT CTA ACC T	101	$t^{w73} > t^{w5} \geq$ C3H ≥ $t^{w12} \geq t^{12}$	1.5/53
Au127	$(CA)_{24}$	ATG AAC TCA CCT ACC CAT GT GCA GAC AGG AAG TTT AGT AC	135	C3H ≥ $t^{w12} > t^{12} > t^{w5}$	1.0/53

[a] $t = t^{12}$, t^{w5}, t^{w12}.

[b] With 10 dinucleotide interruptions.

DNA for 2 hr at 42°. Hybridization of probe was done with the same solution as in the prehybridization except dextran sulfate was added to 7%, and the hybridization was done for 12–24 hr. For cosmid probe preparation insert and vector from minipreps was sonicated to <1 kbp and 100–200 ng random primed to at least 10^8 cpm/μg. Three to five million cpm/ml were added to 200 μg/ml sonicated salmon sperm DNA and 200 μg/ml sonicated cold mouse DNA. This mixture was denatured and incubated for 2 hr at 42° in 8–10 ml hybridization solution, and then added to the blot.

RESULTS

Screening the cell hybrid genomic library: While several methods exist for obtaining random DNA probes for a specific mammalian subregion including microdissection (ROHME *et al.* 1984), PCR amplification of sequences flanking highly repeated elements in somatic cell hybrids (NELSON *et al.* 1989), and screening of genomic libraries derived from somatic cell hybrids with a species-specific repetitive element (KASAHARA, FIGUEROA and KLEIN 1987) only the latter method allows for the quick identification of large fragments of DNA which can be then examined for simple sequence blocks or expressed sequences if desired. Also, the availability of ~40 kbp of insert DNA per clone for use as a probe would help in the mapping of the clones, since this mapping would rely heavily on the detection of RFLPs.

We compared the ability of the highly repetitive interspersed mouse elements B1, Bam5R (FANNING 1982; BENNETT *et al.* 1984; HASTIE 1989) or mouse liver DNA (precompeted with excess cold Chinese hamster DNA) to distinguish between mouse and hamster DNA containing clones. Our results indicated that B1 was relatively ineffective, while Bam5R and mouse liver DNA were both effective and virtually identical in the mouse clones detected (data not shown). A conservative estimate of ~500 copies of Bam5R-related sequences should reside in the *t*-complex (5×10^4 copies/haploid genome × 1%). Hence, for simplicity, Bam5R was the probe of choice. Ninety-one percent (145/159) of the clones thus identified proved to be mouse-DNA-containing clones. The final number of clones screened for map position in the *t*-complex was 173. Of these, 145 were detected with Bam5R and the remaining 28 were added from a previous screen which had used VL30 and IAP as the mouse-specific probe (HOWARD *et al.* 1990).

Mapping to the *t*-complex by RFLP analysis: The goal of the RFLP analysis was to identify which of the 173 cosmid clones would detect RFLPs specific to the *t*-complex when probed against blots of restricted DNA from the inbred strain C3H and from mice carrying the *t*-complex congenic on the C3H background. Since *t*-haplotypes and inbred laboratory mice are thought to have diverged from a common ancestor ~1–2 million years ago (SILVER *et al.* 1987), the use of a small number of restriction enzymes (see MATERIALS AND METHODS) has a reasonable probability of detecting an RFLP between *t*-haplotypes and C3H. In addition, to increase the chances of finding altered enzyme sites between C3H and *t*-chromosomal sequence, the whole cosmid DNA was used as a probe after precompeting the labeled insert with an excess of cold mouse DNA. Hybridization of unique or low copy sequences are not significantly hampered by this method, however, since incorporation of labeled dCTP is relatively low per unit length of template, fragments less than 1 kbp were rarely detected. On

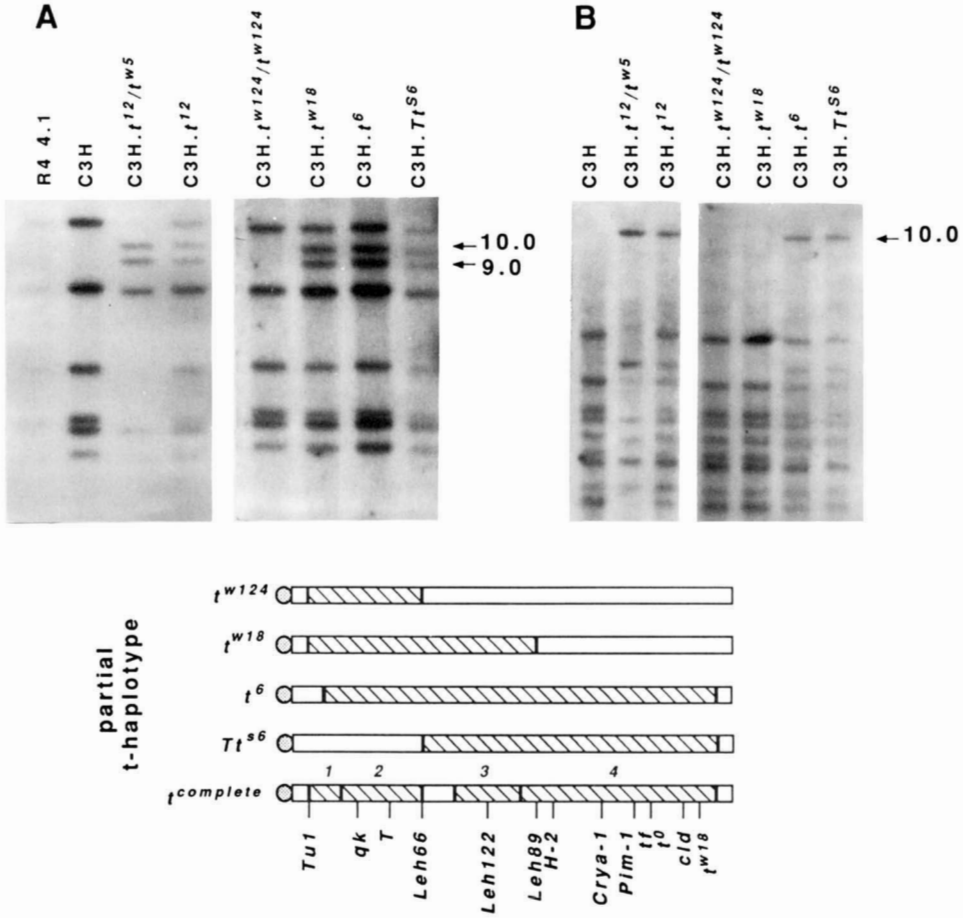

FIGURE 1.—Partial *t*-haplotype mapping. (A) Mapping of *Au144* to the *t* complex. The 9- and 10-kbp *Bam*HI fragments are specific to the lanes carrying the complete *t*-haplotypes (t^{12} and t^{w5}). The R4 4.1 and C3H patterns are identical as expected. The 9- and 10-kbp fragments are not present in t^{w124}/t^{w124} which is wild type for the distal end of the *t*-complex. (B) Mapping of *Au126*. The 10-kbp *Taq*I *t*-type band is shared by the complete *t*-haplotypes t^{w5} and t^{12}. Other smaller *t*-type bands can also be seen. The 10-kbp *t*-type band is present in the t^6 and Tt^{s6} partial *t*-haplotypes only. Precompeted whole cosmid was used as the probe in all hybridizations. Below is a diagram of the four partial *t*-haplotypes; the bottom-most chromosome is a complete *t*-haplotype shown with the four inversions shown above it and relevant markers noted below. Crosshatched portions represent *t*-chromatin.

average, five to seven fragments larger than 1 kbp could be seen with standard 6-bp recognition sequence restriction enzymes. Of 145 Bam5R positive clones, 15 gave an RFLP specific to the *t*-haplotypes t^{12} and t^{w5}. (See Figure 1, A or B, for a typical experiment.) Of 28 VL30/IAP positive clones, two were mapped to the *t*-complex giving a total of 17 *t*-complex DNA markers. More precise mapping of twelve cosmids that map distal to *Leh66B* is described below.

Mapping within the *t*-complex: RFLP analysis was initially used to map 11 of the 12 clones described here into *t*-complex subregions by hybridization to blots containing DNA from partial *t*-haplotypes. (Only one clone, *Au64*, showed an RFLP between *t*-haplotypes; its mapping is discussed below.) Partial *t*-haplotypes arise from rare recombination events between complete *t*-haplotypes and wild-type chromosomes. In these exceptional recombinants, wild-type chromatin has replaced *t*-chromatin in either proximal or distal regions. ("Proximal" or "distal" will refer to the *t*-complex and its relative orientation of markers unless otherwise noted.) The partial *t*-haplotypes used were t^6 and $Tt^{s6}tf$, which have wild-type chromatin at the proximal end, and t^{w18} and $t^{w124}tf$, which have wild-type chromatin at the distal end (see Figure 1). Figure 1A demonstrates a typical mapping experiment using whole cosmid clone 144 as the probe. The two *t*-specific bands at 9- and 10-kbp are present only in t^6, Tt^{s6} and t^{w18}, but not t^{w124}, indicating that t^{w124}, which retains only the proximal end of the *t*-complex, has lost the *t*-specific allele. The two bands are present in t^{w18}, another proximal recombinant, whose breakpoint is further distal than that of t^{w124}. Hence, the locus *Au144* should map to that region of the *t*-complex present in t^{w18}, but not t^{w124}. Cosmids defining the loci *Au17*, *Au31*, *Au49* and *Au53* gave results identical to cosmid 144 and map to the same region. This region has a distal limit in *t*-haplotypes set by the breakpoint near the loci duplicated in the t^{w18} partial *t*-haplotype (*D17Leh89*, *D17Leh467*, *D17Leh525*; BUĆAN *et al.* 1987) and a proximal limit set by t^{w124} at the *Leh66B* locus (HOWARD *et al.* 1990). Since the t^{w18} breakpoint

occured within *In(17)4*, one or more of these new markers may be separated in wild-type to regions more distal, like the loci duplicated in the t^{w18} partial *t*-haplotype.

DNA sequences from a second set of cosmids mapped more distal in *t*-haplotypes; these cosmids define the loci *Au7*, *Au16*, *Au57*, *Au100*, *Au126* and *Au127*. A typical mapping experiment using whole cosmid clone 126 as the probe is shown in Figure 1B. These clones retain their *t*-type band in t^6 and Tt^{s6} only, indicating a map position distal to the t^{w18} breakpoint in *In(17)4*.

Specific map positions of four cosmids: The map positions of four cosmids located within *In(17)4* were refined. $(CA)_n$ length polymorphism (WEBER and MAY 1989) at *Au7*, *Au126* and *Au127* and RFLP analysis at *Au64* were used to identify their respective alleles on a set of anchored inter-*t*-haplotype recombinants with breakpoints in *In(17)4*. For each locus a preliminary survey of the anchored intervals indicated on which interval to focus.

***Au64* and *Au127* set a new distal limit in *t*-haplotypes for the position of the t^{w5} gene:** The P64 probe detects a unique 6.8-kbp *Pst*I band in t^{12} not shared by the other *t*-haplotypes involved in the crosses or by the wild-type *trans* chromosome used. *Au127* contains a $(CA)_n$ block which showed a three-way polymorphism between t^{12}, t^{w5} and Tt^{s6} (see Figure 2). The initial typing showed that these two clones reside in the ~3 cM *H-2K* to *Crya-1* interval. We typed 33 recombinants with breakpoints between these two anchor loci. Fourteen recombinants separated *Au64* from *H-2K*, while 19 separated *Au64* from *Crya-1*. Almost identical results were obtained with the *Au127* locus. Twelve breakpoints mapped between *H-2K* and *Au127* and 21 mapped between *Au127* and *Crya-1*. There were, however, two recombinants with breakpoints in between these markers. These results allowed us to order these loci and estimate genetic distances (cM) in *t*-haplotypes as follows: *cen* . . . *H-2K*/t^{w5} . . (1.1) . . *Au127* . . (0.2) . . *Au64* . . (1.7) . . *Crya-1*.

Closer scrutiny of cosmids 127 and 64 indicated that they are very likely overlapping. First, they share several *Bam*HI, *Eco*RI and *Hin*dIII fragments as judged by inspection of complete restriction enzyme digestion patterns, and secondly, several fragments shared by the two cosmids cross-hybridize following precompetition of the labeled cosmid with cold mouse DNA. One of these cross-hybridizing fragments was isolated and hybridized to genomic blots and appears to be a two-copy sequence devoid of any moderately or highly repeated elements.

Since several of the recombinants derived from the t^{w5} complete *t*-haplotype had been progeny tested for the presence of the t^{w5} lethal mutation, these loci set a limit toward *Crya-1* for the position of t^{w5}. t^{w5} is genetically inseparable from *H-2K*, and these data indicate that it can be no further than ~1.1 cM distal to *H-2K* in *t*-haplotypes.

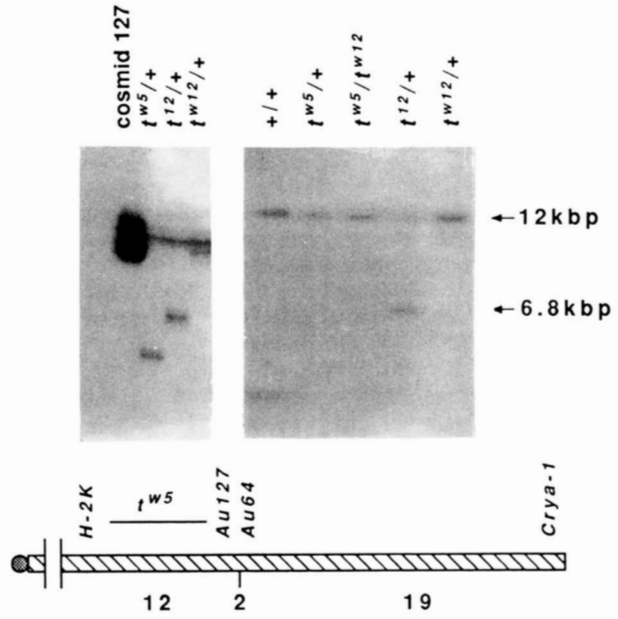

FIGURE 2.—Map position of *Au127* and *Au64*. Top left, $(CA)_n$ polymorphism at *Au127*. Top right, *Pst*I polymorphism detected by P64. Bottom, chromosome showing positions of markers in the region between *H-2K* and *Crya-1* in the *t*-haplotype orientation. Below the chromosome are the number of recombinants detected between markers.

The *Au126* locus maps near the *cld* and t^0 genes distal to *tf*: Results with the whole cosmid 126 probe indicated that the t^{w18} partial proximal *t*-haplotype had lost the *t*-specific fragment seen in other complete *t*-haplotypes (see Figure 1B), indicating that *Au126* must either map in *In(17)4* or be deleted from t^{w18} (BUĆAN *et al.* 1987). However, *Au126* should be present on the t^{w18} haplotype for the following reason: Amplification across a $(CA)_n$ block at the *Au126* locus from a C3H.t^{w18} animal yielded a length polymorphism that could only have come from the wild-type region of the t^{w18} haplotype (see Figure 3C). In other words, even though the t^{w18} haplotype was made congenic on C3H, it still retained a portion of feral chromosome between the end of the *t*-chromatin and the beginning of the C3H-derived chromosome. [The t^{w18} exception occurred in a t^{w5} haplotype still trans to a chromosome from the wild (BENNETT and DUNN 1960).] The feral chromosome and C3H were polymorphic indicating that this locus is not deleted in the t^{w18} partial *t*-haplotype and sets a distal limit for *Au126* in *t*-haplotypes at the t^{w18} deletion.

The $(CA)_n$ block at *Au126* was found to be polymorphic between the parental *t*-haplotypes used in our genetic crosses (see Figure 3C). The preliminary survey of anchored intervals showed that the locus resides distal to the t^{w12} gene in *t*-haplotypes. Four recombinants breaking between t^{w12} and *tf* were available for typing. In no case was *Au126* separated from

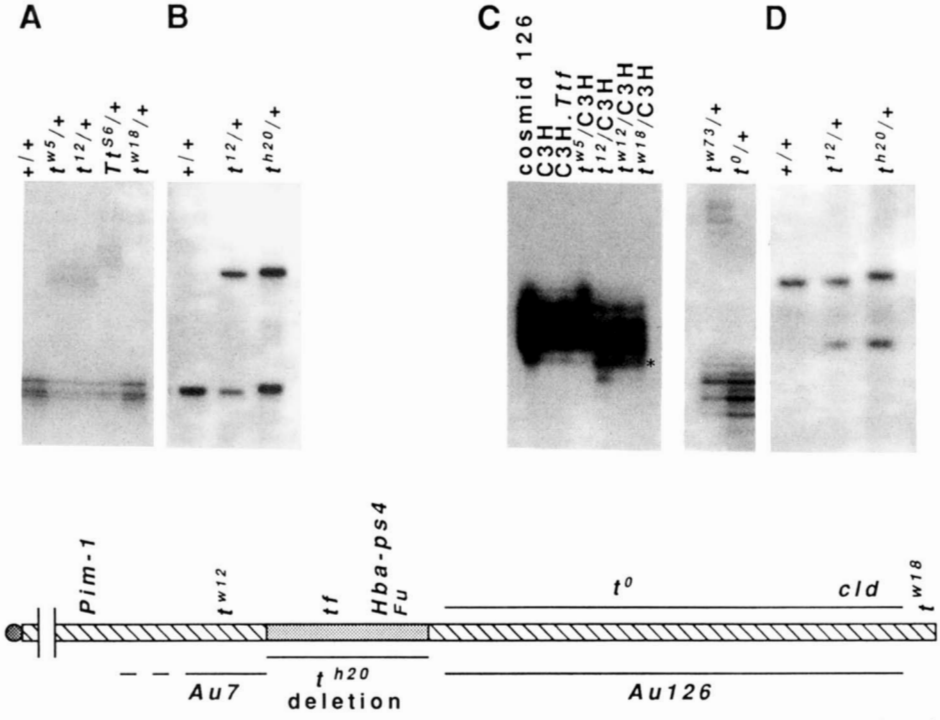

FIGURE 3.—Map position of *Au7* and *Au126*. (A and C) $(CA)_n$ polymorphism at *Au7* and *Au126*, respectively. The polymorphism from the wild-type region of the t^{w18} haplotype is marked with an asterisk in (C). (B and D) Neither *Au7* (probe P7) nor *Au126* (probe P126), respectively, are deleted from t^{h20}. Bottom, chromosome showing position of markers between *Pim-1* and t^{w18} in the *t*-haplotype orientation. Stippled region identifies the deletion in t^{h20}. The position of t^0 proximal to *cld* is inferred from separate genetic crosses and is tentative.

tf. Only two recombinants were available which involved the more distal interval between *tf* and *cld*. In both cases *Au126* mapped with *cld*, distal to *tf*. This placement was corroborated by the t^{h20} haplotype which deletes *Hba-ps4*, *tf* and *Fu* (LYON and BECHTOL 1977; FOX, SILVER and MARTIN 1984) but not *Au126* (see Figure 3D) nor *cld* (LYON 1985).

***Au7* maps near the t^{w12} gene:** A $(CA)_n$ length polymorphism allowed us to initially map this locus distal to *Pim-1* in *t*-haplotypes (see Figure 3A). Analysis of three recombinants breaking between *Pim-1* and t^{w12}, and four recombinants breaking between t^{w12} and *tf* showed that *Au7* was not separated from t^{w12} in these seven events. These are the same recombinants used by ARK *et al.* (1991), to position *Pim-1*, t^{w12} and t^0. The distal limit of *Au7* can be set proximal to *tf* by the t^{h20} deletion, which deletes *tf*, *Hba-ps4* and *Fu*, but not *Au7* (see Figure 3B).

DISCUSSION

We have identified a set of 17 cosmid clones that reside in the *t*-complex and mapped the position of 12 of these clones. Five loci have been mapped in *t*-haplotypes to the interval between *Leh66B* and *Leh89*. Three loci have been broadly mapped to *In(17)4*, and the remaining four loci have been specifically mapped near developmental mutations in *In(17)4* (see Figure 4).

Distribution of the markers: The level of resolution of our mapping does not allow for unequivocal interpretations of chromosomal distribution of the cosmid clones, however, on a gross scale, it appears fairly random. It should be noted, however, that 12 of 15 Bam5R-identified cosmid clones mapped distal to the *Leh66B* locus in *t*-haplotypes while four of four VL30/IAP-identified clones (this number includes two from a previous screen (HOWARD *et al.* 1990)) mapped proximal to *Leh66B*. A similar screen of R4 4.1 derived cosmids using Bam5R plus a second anonymous repetitive element has mapped a high number of clones in or near the major histocompatablity complex (MHC) (KASAHARA, FIGUEROA and KLEIN 1987; VINCEK *et al.* 1989). By contrast, only two of the Bam5R identified loci described here, *Au64* and *Au127*, map close to the MHC. None of the three remaining clones that have been broadly mapped to *In(17)4* cross-hybridize with pH-2IIa, a probe which detects most MHC class I genes.

$(CA)_n$ length polymorphism among *t*-haplotypes: Three of the loci were mapped using $(CA)_n$ length polymorphism and inter-*t*-haplotype crosses. As shown here, these simple sequence repeats are a useful source of polymorphism for genetic mapping in *t*-haplotypes. We actually looked at nine separate $(CA)_n$ blocks in six of the cosmids mapping to *In(17)4* (see MATERIALS AND METHODS). In this small sample size, 33% (3/9) of the $(CA)_n$ blocks identified a polymorphism between at least two *t*-haplotypes. By comparison, 89% (8/9) of the blocks identified length polymorphisms between *t*-haplotypes and the laboratory strains used

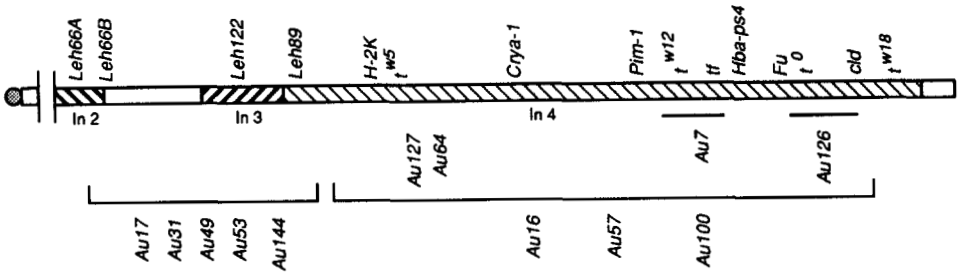

FIGURE 4.—Summary of the map positions of 12 cosmids on a *t*-haplotype. The distal end of *In(17)2* is shown at left. Clear boxes are noninverted regions. The markers described in this paper are noted below the chromosome.

here (C3H or BTBRTF). This difference in polymorphism is comparable to that reported between inbred laboratory mice and *Mus spretus* (CORNALL *et al.* 1991; MONTAGUTELLI, SERIKAWA and GUÉNET 1991).

Markers mapping near developmental mutations: The *Au127* and *Au64* markers map ~1.1 cM distal to *H-2K*. *H-2K* has not been genetically separated from the t^{w5} recessive lethal in over 1200 mice analyzed, and they are therefore thought to be located very close to each other (ABE *et al.* 1988). This notion assumes a random distribution of breakpoints in this region. However, it has been shown that a hotspot of recombination exists within 100 kbp of *H-2K* toward the H-2I region (ARTZT *et al.* 1988), therefore the physical distance between *H-2K* and t^{w5} might be quite different from that implied by the genetic data. The proximal limit of t^{w5} in *t*-haplotypes has been shown to be at a point ~65 kbp toward *H-2Pb* from *H-2K*. Until now the closest distal marker mapped with respect to t^{w5} has been the *Crya-1* gene some 3.0 cM from *H-2K*. The physical size of the region between *H-2K* and the new distal limit, *Au127*, in which the t^{w5} gene must map is unknown. However, using the standard conversion in mice between genetic and physical distance shows that the physical distance may still be quite large, $\sim 2 \times 10^6$ bp.

The t^{w5} gene is an interesting target for cloning. Homozygous mutant embryos are affected in early development at 5–6 days post coitum, when embryonic ectoderm dies (BENNETT 1975). The two new markers that set a distal boundary for the gene should aid in its cloning.

The *Au7* locus has been mapped near the t^{w12} recessive lethal. This mutation affects neural tube and brain development, initially causing the ventral half of both these tissues to become pycnotic and die, as does the entire embryo somewhat later (BENNETT 1975). Interestingly, this mutation distinguishes between the ventral and dorsal halves of the developing nervous system. The mapping of *Pim-1*, t^{w12}, and *tf* by ARK *et al.* (1991), suggests that *Au7* may be very close to t^{w12}. Distances of 0.6 cM from *Pim-1* to t^{w12} and of 0.5 cM from t^{w12} to *tf* were estimated from inter-*t*-haplotype crosses. These same recombinants have been used in this study. In addition, the t^{h20} deletion breakpoint should be proximal to *tf*; this further reduces the interval, since *Au7* is not deleted in t^{h20}.

The *Au126* locus may map near two recessive lethal mutations, one, *cld*, which affects lipid metabolism in neonates (PATERNITI *et al.* 1983), and the second, t^0 (t^6), a mutation which causes the early embryo to fail in the stage when it differentiates into extra-embryonic and embryonic ectoderm (BENNETT, BADENHAUSEN and DUNN 1959). The relative positions of *Fu*, t^0 and *cld* are not firmly established; however, *Fu* is deleted in t^{h20} and *cld* is not. The presence or deletion of the $t^0(t^6)$ gene cannot be ascertained since t^{h20} was derived from the t^6 haplotype. Since *Au126* is not deleted in t^{h20}, we tentatively place it closer to t^0 and *cld* than to *Fu*.

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LITERATURE CITED

ABE, K., J.-F. WEI, F.-S. WEI, Y.-C. HSU, H. UEHARA, K. ARTZT and D. BENNETT, 1988 Searching for coding sequences in the mammalian genome: The *H-2K* region of the mouse MHC is replete with genes expressed in embryos. EMBO J. **7:** 3441–3449.

ADAMSON, E. D., 1987 Oncogenes in development. Development **99:** 449–471.

ARK, B., G. GUMMERE, D. BENNETT and K. ARTZT, 1991 Mapping of the *Pim-1* oncogene in *t*-haplotypes and its use to define the relative map positions of the *tcl* loci $t^0(t^6)$ and t^{w12} and the marker *tf* (*tufted*). Genomics **10:** 385–389.

ARTZT, K., 1984 Gene mapping within the *T/t* complex of the mouse. III. *t*-Lethal genes are arranged in three clusters on chromosome 17. Cell **39:** 565–572.

ARTZT, K., B. BABIARZ and D. BENNETT, 1979 A *t*-haplotype (t^{w75}) overlapping two complementation groups. Genet. Res. **33:** 279–285.

ARTZT, K., K. ABE, H. UEHARA and D. BENNETT, 1988 Intra-*H-2* recombination in *t*-haplotypes shows a hot spot and close linkage of l^{w5} to *H-2K*. Immunogenetics **28:** 30–37.

BABIARZ, B. S., 1983 Deletion mapping of the *T/t* complex: evidence for a second region of critical embryonic genes. Dev. Biol. **95:** 342–351.

BENNETT, D., 1975 The *T*-locus of the mouse. Cell **6:** 441–454.

BENNETT, D., S. BADENHAUSEN and L. C. DUNN, 1959 The embryological effects of 4 late lethal *t*-alleles in the mouse which affect the neural tube and skeleton. J. Morphol. **105:** 105–144.

BENNETT, D., and L. C. DUNN, 1960 A lethal mutation (t^{w18}) in the house mouse showing partial duplications. J. Exp. Zool. **143:** 203–219.

BENNETT, K. L., R. E. HILL, D. F. PIETRAS, M. WOODWORTH-

GUTAI, C. KANE-HAAS, J. M. HOUSTON, J. K. HEATH and N. D. HASTIE, 1984 Most highly repeated dispersed DNA families in the mouse genome. Mol. Cell. Biol. **4:** 1561–1571.

BUĆAN, M., B. G. HERRMANN, A.-M. FRISCHAUF, V. L. BAUTCH, V. BODE, L. M. SILVER, G. R. MARTIN and H. LEHRACH, 1987 Deletion and duplication of DNA sequences is associated with the embryonic lethal phenotype of the t^9 complementation group of the mouse *t*-complex. Genes Dev. **1:** 376–385.

Committee on the Mouse Chromosome *17*, 1991 Maps of mouse chromosome *17*: first report. Mamm. Genome **1:** 5–29.

CORNALL, R. J., T. J. AITMAN, C. M. HEARNE and J. A. TODD, 1991 The generation of a library of PCR-analyzed microsatellite variants for genetic mapping of the mouse genome. Genomics **10:** 874–881.

EBERSOLE, T., O. RHO and K. ARTZT, 1992 The proximal end of mouse chromosome *17*: new molecular markers identify a deletion associated with *quaking*^{viable}. Genetics **131:** 183–190.

FANNING, T. G., 1982 Characterization of a highly repetitive family of DNA sequences in the mouse. Nucleic Acids Res. **10:** 5003–5013.

FOX, H. S., L. M. SILVER and G. R. MARTIN, 1984 An alpha globin pseudogene is located within the mouse *t* complex. Immunogenetics **19:** 125–130.

FRISCHAUF, A.-M., 1985 The *T/t* complex of the mouse. Trends Genet. **1:** 100–102.

GARDINER, K., M. HORISBERGER, J. KRAUS, U. TANTRAVAHI, J. KORENBERG, V. RAO, S. REDDY and D. PATTERSON, 1990 Analysis of human chromosome *21*: correlation of physical and cytogenetic maps; gene and CpG island distributions. EMBO J. **9:** 25–34.

GOSSLER, A., A. L. JOYNER, J. ROSANT and W. C. SKARNES, 1989 Mouse embryonic stem cells and reporter constructs to detect developmentally regulated genes. Science **244:** 463–465.

GRAHAM, A., N. PAPALOPULU and R. KRUMLAUF, 1989 The murine and *Drosophila* homeobox gene complex have common features of organization and expression. Cell **57:** 367–378.

HAMMER, M. F., J. SCHIMENTI and L. M. SILVER, 1989 Evolution of mouse chromosome *17* and the origin of inversions associated with *t*-haplotypes. Proc. Natl. Acad. Sci. USA **86:** 3261–3265.

HASTIE, N. D., 1989 Highly repeated DNA families in the genome of *Mus musculus*, pp. 559–573 in *Genetic Variants and Strains of the Laboratory Mouse*, Ed. 2, edited by M. F. LYON and A. G. SEARLE. Oxford University Press, Oxford.

HERRMANN, B., M. BUĆAN, P. E. MAINS, A.-M. FRISCHAUF, L. M. SILVER and H. LEHRACH, 1986 Genetic analysis of the proximal portion of the mouse *t*-complex: evidence for a second inversion within *t*-haplotypes. Cell **44:** 469–476.

HERRMANN, B. G., S. LABEIT, A. POUSTKA, T. R. KING and H. LEHRACH, 1990 Cloning of the *T* gene required in mesoderm formation in the mouse. Nature **343:** 617–622.

HOWARD, C. A., G. R. GUMMERE, D. BENNETT and K. ARTZT, 1990 Genetic and molecular analysis of the proximal region of the mouse *t*-complex using new molecular probes and partial *t*-haplotypes. Genetics **126:** 1103–1114.

JAKOBOVITS, A., G. M SHACKLEFORD, H. E. VARMUS and G. R. MARTIN 1986 Two proto- oncogenes implicated in mammary carcinogenesis, *int-1* and *int-2*: are independently regulated during mouse development. Proc. Natl. Acad. Sci. USA **83:** 7806–7810.

KASAHARA, M., F. FIGUEROA and J. KLEIN, 1987 Random cloning of genes from mouse chromosome *17*. Proc. Natl. Acad. Sci. USA **84:** 3325–3328.

KESSEL, M., and P. GRUSS, 1990 Murine developmental control genes. Science **249:** 374- 379.

LYON, M. F., 1984 Transmission ratio distortion in mouse *t*-haplotypes is due to multiple distorter genes acting on a responder locus. Cell **37:** 621–628.

LYON, M. F., 1985 Private communication. Mouse News Letter. **72:** 104.

LYON, M. F., 1986 Male sterility of the mouse *t*-complex is due to homozygosity of the distorter genes. Cell **44:** 357–363.

LYON, M. F., and K. B. BECHTOL, 1977 Derivation of mutant *t*-haplotypes of the mouse by presumed duplication or deletion. Genet. Res. **30:** 63–76.

MONTAGUTELLI, X., T. SERIKAWA and J.-L. GUÉNET, 1991 PCR-analyzed microsatellites: data concerning laboratory and wild-derived mouse inbred strains. Mamm. Genome **1:** 255–259.

NELSON, D., S. A. LEDBETTER, L. CORBO, M. F. VICTORIA, R. RAMIREZ-SOLIS, T. D. WEBSTER, D. H. LEDBETTER and C. T. CASKEY, 1989 *Alu* polymerase chain reaction: a method for rapid isolation of human-specific sequences from complex DNA sources. Proc. Natl. Acad. Sci. USA **86:** 6686–6690.

PATERNITI, J. R., W. V. BROWN, H. N. GINSBERG and K. ARTZT, 1983 Combined lipase deficiency (*cld*): A lethal mutation on chromosome *17* of the mouse. Science **221:** 167–169.

ROHME, D., H. FOX, B. HERRMANN, A. FRISCHAUF, J. EDSTROM, P. MAINS, L. SILVER and H. LEHRACH, 1984 Molecular clones of the mouse *t*-complex derived from microdissected metaphase chromosomes. Cell **36:** 783–788.

SCHÖLER, H., G. R. DRESSLER, R. BALLING, H. ROHDEWOHLD and P. GRUSS, 1990 *Oct-4*: a germline-specific transcription factor mapping to the mouse *t*-complex. EMBO J. **9:** 2185–2195.

SHIN, H.-S., L. FLAHERTY, K. ARTZT, D. BENNETT and J. RAVETCH, 1983 Inversion in the *H-2* complex of *t*-haplotypes in mice. Nature **306:** 380–383.

SILVER, L. M., and K. ARTZT, 1981 Recombination suppression of mouse *t*-haplotypes due to chromatin mismatching. Nature **290:** 68–70.

SILVER, L. M., and D. REMIS, 1987 Five of the nine genetically defined regions of mouse *t*-haplotypes are involved in transmission ratio distortion. Genet. Res. **49:** 51–56.

SILVER, L. M., M. HAMMER, H. FOX, J. GARRELS, M. BUCAN, B. HERRMANN, A.-M. FRISCHAUF, H. LEHRACH, H. WINKING, F. FIGUEROA and J. KLEIN, 1987 Molecular evidence for the rapid propagation of mouse *t*-haplotypes from a single, recent, ancestral chromosome. Mol. Biol. Evol. **4:** 473–482.

SMILEY, J. R., D. A. STEEGE, D. K. JURICEK, W. P. SUMMERS and F. H. RUDDLE, 1978 A herpes simplex virus 1 integration site in the mouse genome defined by somatic cell genetic analysis. Cell **15:** 455–468.

VINCEK, V., H. KAWAGUCHI, K. MIZUNO, Z. ZALESKA-RUTCZNSKA, M. KASAHARA, J. FOREJT, F. FIGUEROA and J. KLEIN, 1989 Linkage map of chromosome *17*: localization of 27 new DNA markers. Genomics **5:** 773–786.

WEBER, L. M., and P. E. MAY, 1989 Abundant class of human DNA polymorphism which can be typed using the polymerase chain reaction. Am. J. Hum. Genet. **44:** 388–396.

ZIMMER, A., and P. GRUSS, 1989 Production of chimaeric mice containing embryonic stem (ES) cells carrying a homeobox *Hox 1.1* allele mutated by homologous recombination. Nature **338:** 150–156.

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