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The Proximal End of Mouse Chromosome *17*: New Molecular Markers Identify a Deletion Associated With *quaking^{viable}*

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ABSTRACT

Five randomly identified cosmids have been mapped proximal to the *Leh66D* locus on mouse chromosome *17*. Two of these cosmids, *Au10* and *Au119*, map near the neurological mutation *quaking*. *Au119* is deleted in qk^{viable}/qk^{viable} DNA, whereas *Au10* is not. *Au76* maps to a gene-rich region near the *Tme* locus. The *Au76* locus encodes a member of a low copy gene family expressed in embryos, the adult central nervous system and testis. A second member of this family has been mapped to chromosome *15* near *c-sis* (*PDGF-B*). At the centromeric end of chromosome *17*, *Au116* maps near the *Tu1* locus, and along with *Au217rs* identifies a region of unusually high recombinational activity between *t*-haplotypes and wild-type chromosomes. *Au217I* and *II* map to the large inverted repeats found at the proximal end of the wild-type chromosome. In addition, the *Au217I* and/or *II* loci encode testis transcripts not expressed from *t*-haplotypes.

THE attention given the proximal third of mouse chromosome *17* derives, in large part, from the analysis of *t*-haplotypes, a variant form found in wild populations of mice. *t*-Haplotypes exhibit a set of well defined characteristics: (1) recombination suppression with wild-type chromosomes over a region of an estimated 40-Mbp, a consequence of the existence of several large inversions (Hammer, Schimenti and Silver 1989; Herrmann *et al.* 1986; Shin *et al.* 1983); (2) transmission ratio distortion favoring *t*-haplotype-bearing sperm in *t*/+ heterozygous males; (3) sterility of t^x/t^y males; and (4) usually the presence of one or more recessive lethal mutations (Bennett 1975).

The region encompassed by the inversions is called the *t*-complex. The area within the two most proximal inversions, *In(17)1* and *In(17)2*, are referred to as the "proximal end." This region is delimited in wild-type chromosomes by the markers *Tu1* and *Leh66D*. The proximal end of *t*-haplotypes has been compared with its wild-type counterpart in terms of chromosome structure and apart from the inversions, a notable structural difference involves an element of at least 650 kbp existing in one copy in *t*-haplotypes which is duplicated and inverted in wild-type chromosomes. These elements are thought to serve as regions of matched chromatin which can yield rare recombination events between *t*-haplotypes and wild-type chromosomes (Herrmann, Barlow and Lehrach 1987). The resulting partial *t*-haplotypes have been valuable mapping tools for the region.

With the use of partial *t*-haplotypes, deletions occurring in wild-type chromosomes or *t*-haplotypes, and classical recombination analysis, many markers have been mapped into the proximal end. The distribution of markers is, however, quite variable. The highest densities occur in the ~3-Mbp interval between *Leh119I* and *T* (>3 markers/Mbp), and in the ~1-Mbp *Plg* to *Leh66D* interval (>6 markers/Mbp). By comparison, the regions between *T* and *Plg*, and proximal to *Leh119I* are relatively poorly marked (see Figure 1). The combined physical distance involved in these underrepresented regions can only be estimated, but a conservative minimum distance would be ~10 Mbp, which would convert to less than one marker per 10^6 bp.

In order to enhance the genetic and physical maps of the proximal end of chromosome *17* we report the mapping of unique or low copy sequences from five cosmids centromeric to the *Leh66D* locus in wild-type chromosomes. These cosmids are part of a larger set mapping to the *t*-complex. They were identified by screening a Chinese hamster-mouse cell hybrid genomic library with mouse-specific repetitive element probes, followed by mapping of mouse-derived cosmids to the *t*-complex using *t*-haplotype *vs.* wild-type restriction fragment length polymorphism (RFLP) analysis (Ebersole, Lai and Artzt 1992). The data presented here introduces new markers into underrepresented regions of chromosome *17* proximal to *Leh66D*. Furthermore, these new markers have allowed us to confirm previously described chromosome structure, identify a region of unusual recombinational activity between *t*-haplotypes and wild-type chromosomes, and show that the original spontaneous *qk* mutation (Sidman, Dickie and Appel 1964) is

associated with a deletion. Also included in this report are the map positions of some members of two low copy gene families detected with these cosmids. One family, from a locus duplicated on wild-type chromosome *17* (loci: *D17Au217I* and *D17Au217II*), encodes two testis transcripts not expressed in *t*-haplotypes. The other family, a member of which is found at the *D17Au76* locus, encodes transcripts found in testicular germ cells, early embryos, and, in later embryos and adults, in restricted parts of the central nervous system.

MATERIALS AND METHODS

DNA and probe sources: Characterization of the breakpoints of the partial *t*-haplotypes t^6 and t^{ae5}, and of the deletion chromosomes T^{hp}, T^{Or}, T^{22H}, Tt^{Orl} and Tt^{Lub2} are found in Committee on the Mouse Chromosome *17* (1991). The partial *t*-haplotypes t^{w5x} and $t^{w111}tf$ are described in HOWARD *et al.* (1990). Additional references for some chromosomes follow: T^{hp}, JOHNSON (1974); T^{Or}, SELBY (1973); also see BENNETT *et al.* (1975); BABIARZ (1983); Tt^{Orl}, SARVETNICK *et al.* (1986); t^{ae5}, VOJTISKOVA *et al.* (1976); t^{Lub2}, WINKING and SILVER (1984); t^6, HERRMANN, BARLOW and LEHRACH (1987). DNA was prepared from liver using standard methods or from spleen in 0.5% low-melting-point agarose plugs. Wild type refers either to the C3H/DiSn or BTBRTF/Nev mouse strains. Identification of the five cosmids is reported in a companion study (EBERSOLE, LAI and ARTZT, 1992). Probes for the new loci described here were derived from genomic fragments from cosmid clones or, in the case of P217c, cDNA isolated using the cosmid clone 217. The 1.4-kbp cDNA (*D15Au76rse*) was detected in a testicular cell cDNA library using pooled *Bgl*II fragments from the chromosome *17* cosmid 76. The cDNA, when hybridized back to cosmid 76, will cross-hybridize to it and detect a 1.7-kbp *Bam*HI fragment (P76). All blots were washed under stringent conditions: 0.1 × SSC, 0.1% sodium dodecyl sulfate (SDS), 65°, 10–15 min final wash.

Nomenclature: Because all the probes derive from mouse chromosome *17*, for simplicity, each locus will be referred to by an abbreviation of the formal name, hence *D17Au10* will be called *Au10*, etc. Loci on chromosomes other than *17* will be referred to with the full name. The original *qk* mutation has been renamed qk^{viable} to distinguish it from the recessive lethal ethylnitrosurea (ENU)-induced alleles (KING and DOVE 1991).

The probes for each locus are named with a P followed by the locus name. P10 is a 4.2-kbp *Bam*HI genomic fragment from locus *Au10*; P116, a 2.8-kbp *Hin*dIII genomic fragment; P119, a 8.0-kbp *Eco*RI genomic fragment; P76, a 1.7-kbp *Bam*HI genomic fragment; P217a, a 5.0-kbp *Eco*RI genomic fragment; P217b, a 3.8-kbp *Eco*RI genomic fragment; and P217c, a 920-bp *Eco*RI cDNA fragment isolated from a mouse 10.5-day embryo cDNA library.

Electrophoresis, blotting and hybridizations: Genomic digestions of 8–10 μg of DNA were electrophoresed in TPE buffer through a 0.7% agarose gel for 15–17 hr at ~0.7 V/cm. Transfer to Hybond-N membranes (Amersham) was accomplished by standard capillary blotting techniques. Hybridizations 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% SDS, 1 mM EDTA, with 200 μg/ml sonicated salmon sperm DNA for 2 hr at 42°. Hybridization of probe was done with the same solution as in prehybridization except dextran sulfate had been added to 7%, and hybridization was continued for 12–24 hr. A 100–200-ng sample of probe DNA was random primed to at least 10^8 cpm/μg. Three to four million cpm/ml were added to 200 μg/ml sonicated salmon sperm DNA, denatured and added to the bag. For probes containing repetitive sequences, 100–200 μg/ml sonicated cold mouse DNA were also added, the mixture was denatured and incubated for 1–2 hr at 42° in 8–10 ml hybridization solution, and then added to the bag. All blots were washed twice in 2 × SSC, 0.1% SDS at 65°, followed by a final wash in 0.1 × SSC, 0.1% SDS at 65°.

RESULTS

***Au10* and *Au119* are two loci near *qk*:** The probes P10 and P119 both detect polymorphisms between wild type and *t*-haplotypes with *Taq*I. P10 detects a 5.0-kbp or 3.8-kbp wild-type band, depending on the strain. In *t*-haplotypes a 2.6-kbp band is present and also a faint doublet at about 1.2 kbp (Figure 2). The wild-type bands are missing in T^{hp} and Tt^{Orl}, but not T^{Or} or T^{22H} (refer to Figure 1 for a depiction of these chromosomes). Furthermore, the locus is duplicated in t^{Lub2}, that is, Tt^{Orl}/t^{Lub2} DNA has both wild-type and *t*-type bands, whereas Tt^{Orl} carries neither. An identical situation exists with P119: wild-type bands are deleted in T^{hp} and Tt^{Orl}, but not T^{Or} or T^{22H}, and the locus is duplicated in t^{Lub2} (data not shown). The *D17RP17* locus has been previously mapped to this same region using the same chromosomes (HERRMANN *et al.* 1986; MANN, SILVER and ELLIOTT 1986). The *qk* locus is known to be deleted in Tt^{Orl} and T^{hp}, but not T^{Or} (BENNETT *et al.* 1975; ERICKSON, LEWIS and SLUSSER 1978), and it has been mapped within 0.3 cM of *D17RP17* (KING and DOVE 1991). We, therefore, expected *Au10* and *Au119* to map near *qk* and be useful in the cloning of the gene affected in this interesting neurological mutation. We tested these two loci in quaking mice by hybridization of precompeted whole cosmid to restriction enzyme digests of qk^v/qk^v liver DNA. As shown in Figure 3, the *Au119* cosmid detects several bands in control lanes but not any in qk^v/qk^v DNA. This indicates that most if not all of the ~40-kbp *Au119* cosmid is deleted in qk^v/qk^v mice. This result was verified using two other enzymes and two other DNA preparations, one from spleen and the other from liver. The *Au10* cosmid detected bands in all lanes of the same blot and is outside the deleted region (data not shown).

***Au76* maps near a gene cluster:** With *Msp*I, P76 detects an RFLP between C3H (1.7-kbp) and *t*-haplotypes (3.8-kbp and fainter 4.0-kbp bands) (Figure 4). The wild-type band is missing in T^{hp}, but present in T^{Or} and T^{22H}. Tt^{Orl} is duplicated for the locus, having both band types, while t^{Lub2} is deleted for the *t*-haplotype band. This suggests that *Au76* maps to the same gene-rich region as *Plg*, *Sod-2*, t^{w73}, *Tcp-1*, *Tme*, *Igf2r*, and near the closely linked genes *Tcp10 b* and *c*, *Tcte-2* and *Hst-1* (Committee on the Mouse Chromosome

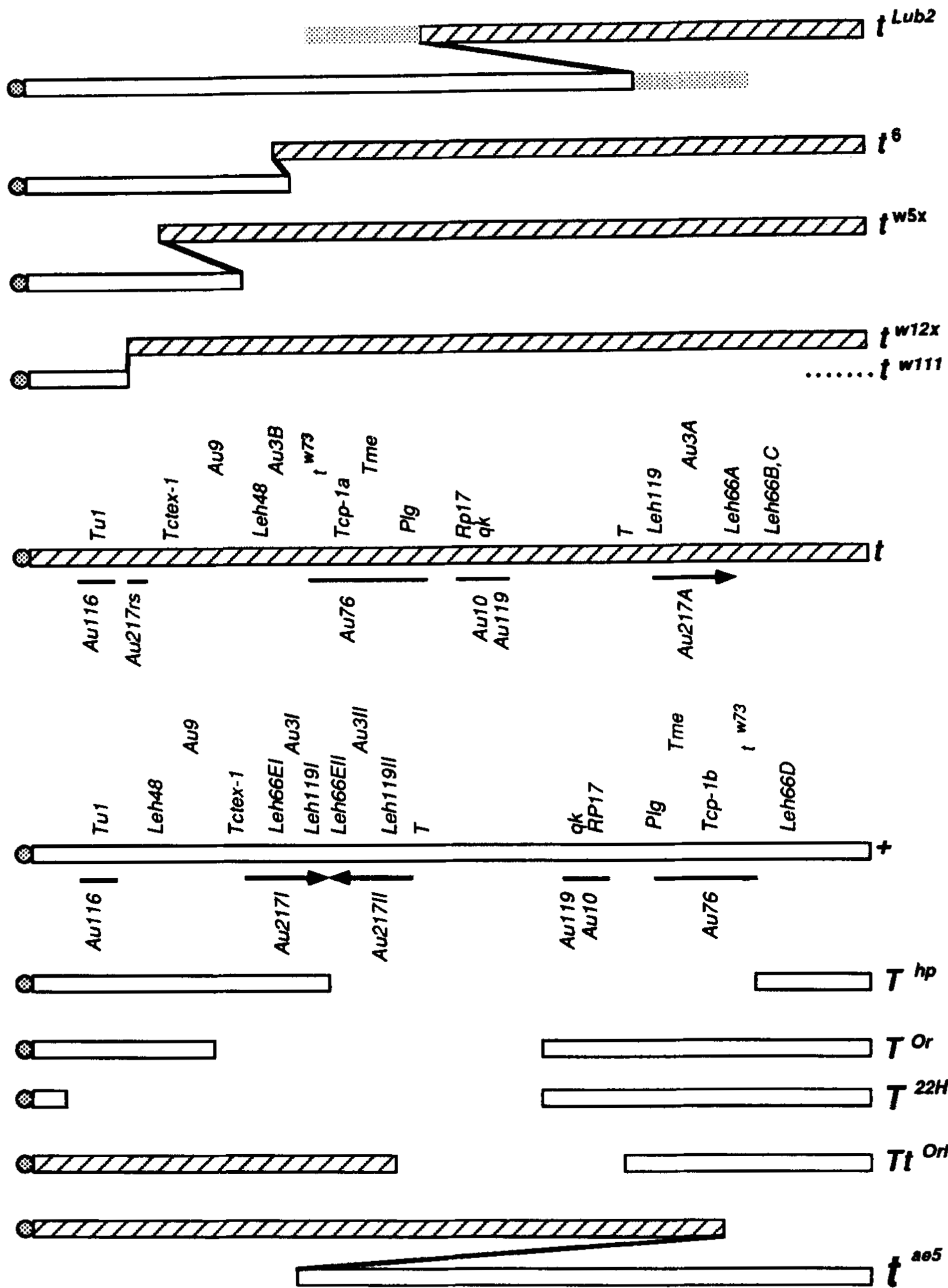

FIGURE 1.—Chromosomes used for mapping proximal to *Leh66B*. Clear boxes indicate wild-type chromatin, crosshatched boxes indicate *t*-chromatin. The stippled boxes in t^{Lub2} are regions known to be deleted in this chromosome. The markers described in this report are noted below the two middle chromosomes. The relative order of markers in the first tier above the chromosomes are known. The position of markers in the upper tier are not defined with respect to neighboring loci. The breakpoint of the t^{w12x} and t^{w111} chromosomes at the proximal end have not been distinguished. The line of dots next to t^{w111} indicates that this chromosome is wild type distal to *Leh66A*.

17 1991; BARLOW *et al.* 1991; FOREJT *et al.* 1991).

The *Au76* locus also potentially encodes a member of a small gene family. A 1.4-kbp testicular cell cDNA identified with genomic fragments from cosmid 76 detects a 2.8-kbp message in adult testis, and a 3.0-kbp message in adult brain and in an embryonic carcinoma cell line (Figure 5). *In situ* hybridization of 6.5 to 18.5 days post coitum embryos shows that message is present in all stages, but becomes confined mainly to the developing central nervous system in late stages (data not shown).

Although the 1.4-kbp cDNA cross-hybridizes strongly with cosmid 76, it proved not to map to chromosome *17*. This cDNA detects four *Bam*HI fragments in C3H DNA, but only one of these is found in the somatic cell hybrid, R4 4.1, which contains only mouse chromosome *17* and *18* (data not shown). The 1.4-kbp cDNA was mapped using a *Taq*I RFLP between C57BL/6 and DBA/2J mice and the BXD RI strains. The parental strains share several *Taq*I fragments, however DBA/2J contains 1.9- and 2.1-kbp bands not present in C57BL/6 (data not shown). Of 22 informative RI lines 20 were concordant with *c-sis* (*PDGF-B*) on chromosome *15* (see Figure 6). Our data give a distance of 2.6 ± 2.05 cM between *c-sis* and this new locus, named *D15Au76rse* (*r*elated *s*equence *e*xpressed). In an independent analysis using an interspecific backcross, no recombination was detected between these loci in 158 animals, which maps this cDNA no more than 1.2 cM from *c-sis* (95% confidence level) (M. J. JUSTICE, personal communication).

Comparison of the sequence of the 1.4-kbp cDNA

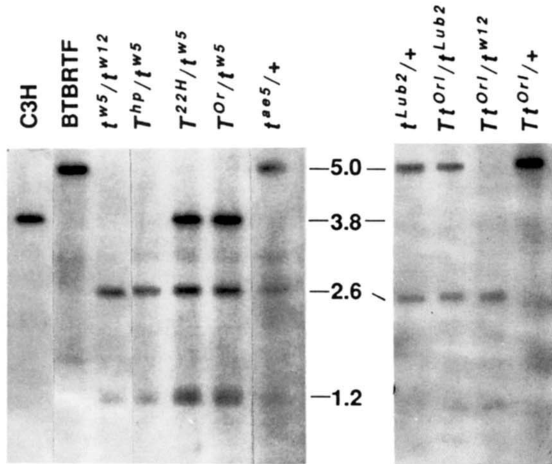

FIGURE 2.—Mapping of the *Au10* locus. *Taq*I-digested DNA hybridized with P10. T^{hp} and Tt^{Orl} delete this locus. It is duplicated in t^{Lub2}.

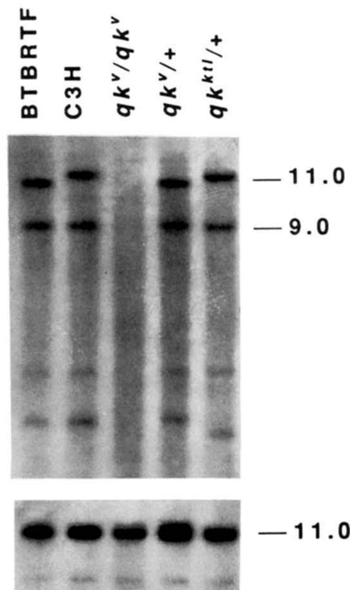

FIGURE 3.—Deletion of the *Au119* locus in qk^v/qk^v DNA. Above, the entire cosmid clone 119 was used as the probe on *Eco*RI-digested DNA after precompeting away repetitive elements. Below, a control hybridization to the same blot with probe P76 detects a high molecular mass fragment (11 kbp) in all lanes. qk^{kt1} is an ENU-induced allele on a *t*-haplotype background.

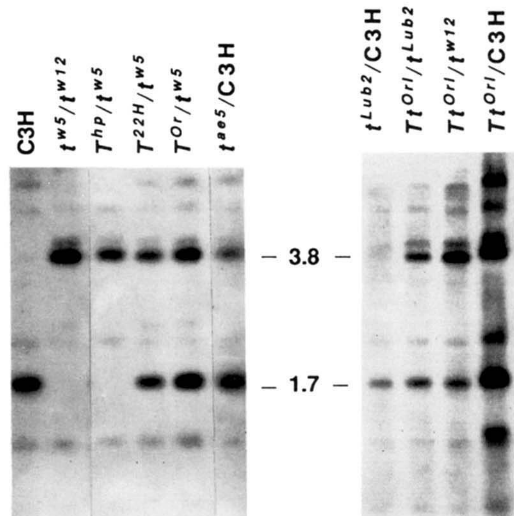

FIGURE 4.—Mapping of the *Au76* locus. Hybridization of *Msp*I-digested DNA with probe P76. T^{hp} deletes this locus. Tt^{Orl} has both wild-type and *t*-type bands. t^{Lub2} deletes the *t*-type band at least, and probably the wild-type one as well. Since *Msp*I (recognition site = CCGG) is sensitive to methylation at the first cytosine, numerous faint bands can be seen.

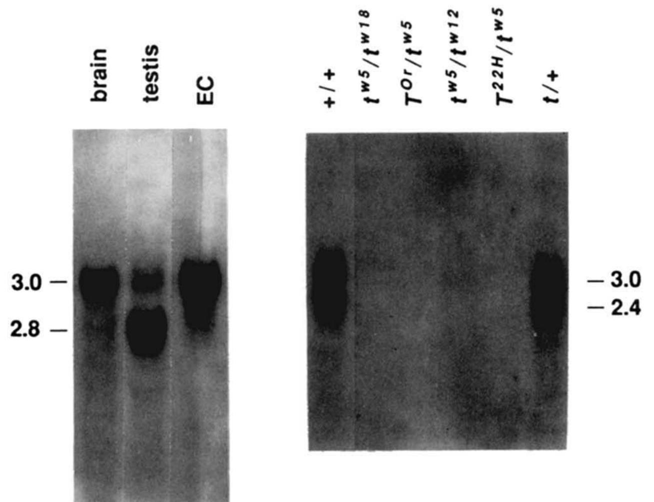

FIGURE 5.—Transcripts encoded at *D15Au76rse* or *Au217I/II*. Left, the 1.4-kbp cDNA from *D15Au76rse* hybridized to brain, testis and embryonal carcinoma (EC) cell total RNA. Right, lanes from a single blot showing the P217c cDNA probe hybridized to relevant testis RNA. The blot was treated with RNase A. t^x/t^y, T^{22H}/t^{w5}, and T^{Or}/t^{w5} RNA lack a detectable transcript. Inspection of the stained gel and a pre-RNase A autoradiogram showed that intact RNA had transferred to the blot from all lanes (data not shown).

(locus: *D15Au76rse*) and the strongly cross-hybridizing 1.7-kbp *Bam*HI genomic fragment (probe P76) from chromosome *17* identified a region of several hundred basepairs with 90% sequence similarity. P76 will detect the same set of transcripts on a Northern as described above for the 1.4-kbp cDNA. When probe P76 is hybridized to *Bam*HI-digested mouse DNA and washed under stringent conditions, four different-sized bands are evident (data not shown). It is, therefore, possible that there are four closely related members in the gene family: one on chromosome *17* in the *Plg* to *Leh66D* interval, one on chromosome *15* near *c-sis*, and two of unknown map position. The relationship among the family members is unexplained beyond the small sequence homology we identified.

***Au116* is at the centromeric end of the *t*-complex:.** The P116 probe is polymorphic with *Eco*RI between C3H (9.6 kbp), and the haplotypes t^{w5} and t^{w12} (4.3 kbp), and t^{12} and t^{ae5} (13.0-kbp) (Figure 7). The locus is deleted in T^{22H}, but not T^{hp} or T^{Or} which retain the 9.6-kbp wild-type band. This suggests a map position near *Tu1*, *Au9*, and *Leh48*. Surprisingly, the partial *t*-haplotype, t^{w111} shows no t^{12}-type 13.0-kbp band, al-

B X D RI Strain

	1	2	5	6	8	9	11	12	13	14	15	16	18	19	20	21	22	23	24	25	27	28	29	30	31	32
D15Au76rse	-	D	D	B	D	D	B	B	D	D	D	B	D	D	-	D	D	-	D	B	D	B	D	D	B	D
c-sis	B	D	D	B	D	D	B	B	D	D	-	B	D	D	D	D	D	B	D	B	D	B	D	D	D	B

FIGURE 6.—Strain distribution pattern detected by the 1.4-kbp cDNA from *D15Au76rse* and by *c-sis* in 26 strains of the B × D RI set. Only strains 31 and 32 are discordant.

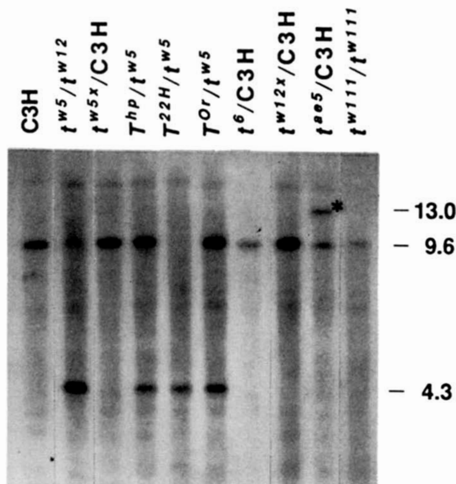

FIGURE 7.—Mapping of the *Au116* locus. *Eco*RI digest of relevant DNA hybridized with probe P116. Neither t^{w5x} nor t^{w12x} have the 4.3-kbp band their "complete" *t*-haplotype counterparts carry. t^{ae5} carries a 13.0-kbp band (asterisk) also found in t^{12} and related *t*-haplotypes, of which t^{w111} is one, but t^{w111} has lost proximal *t*-chromatin. The 9.6-kbp wild-type band is deleted in T^{22H}.

though t^{12} was the parental *t*-haplotype in the crossover event that generated it. The presence of only a wild-type band is evidence for a second recombination event very proximal in *t*-chromatin. P116 detects a similar event in the t^{w5x} chromosome and in another chromosome (now extinct) segregating in our t^{w12} stock which was represented in our DNA collection. We have renamed this chromosome t^{w12x} by analogy with t^{w5x}. In both cases only the wild-type band is present.

The t^{w5x} chromosome has been examined previously at the *Tu1* locus, which maps proximal to *In(17)1* (HOWARD *et al.* 1990). t^{w5x} is known to have lost *t*-chromatin at *Tu1*, thus, we analyzed the *Tu1* locus in t^{w12x} and t^{w111} and found that they too have lost the *t*-specific bands at *Tu1* (data not shown). Both t^{w111} and t^{w12x} retain *t*-chromatin around the *Tctex-1* locus (data not shown) and at *Au217rs* (see below). This data suggests that *Au116* maps proximal to *Tctex-1* and *Au217rs* in *t*-haplotypes, in the vicinity of *Tu1*.

***Au217* maps to the large inverted repeats:** The mapping of *Au217* is based on the cumulative data for two genomic probes, P217a and P217b, subcloned from cosmid 217, and a cDNA probe, P217c isolated from a 10.5-day embryo cDNA library using whole cosmid DNA as the probe. The two genomic probes indicate that there are two copies of the *Au217* locus in wild-type chromosomes (loci: *Au217I* and *Au217II*) and only one copy in *t*-haplotypes (locus: *Au217A*). However, mapping of the P217c cDNA probe in *t*-haplotypes indicates that an additional copy hybridizing to the cDNA sequence exists uniquely in *t*-haplotypes at a very proximal position (locus: *Au217rs*, (*rs*, *r*elated *s*equence)). The evidence is as follows: P217a detects 5.0-kbp and 2.6-kbp wild-type bands and 2.9-, 2.6- and 2.2-kbp *t*-haplotype bands with *Hin*dIII (Figure 8A). P217b detects 1.8- and 1.6-kbp wild-type bands and a 3.8-kbp *t*-haplotype band with *Hin*dIII (Figure 8B). In T^{Or}/t^{w5} DNA only the P217a 5.0-kbp wild-type band is missing while with P217b both wild-type bands are absent. This indicates that the two wild-type loci are deleted in T^{Or} (the P217a 2.6-kbp band is from t^{w5}). These two wild-type loci are probably not extremely close to one another since they are separated by T^{hp}, which deletes the P217b 1.6-kbp band, but not the 1.8-kbp band. Although P217a does not show any missing bands in T^{hp}/t^{w5} DNA, we presume that the 2.6-kbp band, which is shared by *t*-haplotypes and wild type, is from t^{w5} only, the wild-type fragment being deleted in T^{hp}. The distal wild-type copy of *Au217* should reside in the deleted region shared by T^{Or} and T^{hp}. The proximal wild-type copy should map to DNA present in T^{hp} and deleted in T^{Or}.

t-haplotypes carry one copy of the genomic probes P217a and P217b. We beleive that the *Au217* genomic locus maps to the two inverted repeats found in wild-type chromosomes and to the single copy of the region in *t*-haplotypes. This structural difference between *t*-haplotypes and wild-type chromosomes has been previously characterized using the Tu119, Tu66 and Au3 probes (HERRMANN, BARLOW and LEHRACH 1987; HOWARD *et al.* 1990). However, suprising results were obtained with the P217c cDNA probe. Similar to the results with genomic probes P217a and b, P217c detects related sequences at *Au217I* and *II* in wild-type chromosomes and at *Au217A* in *t*-haplotypes. In contrast, P217c detects additional copies of itself that map very proximal in *t*-haplotypes, because the 2.9 *Taq*I *t*-haplotype band detected by P217c is missing in t^6 and t^{w5x} (Figure 8C). This suggests a transposition-like event carried the transcribed sequence, but not

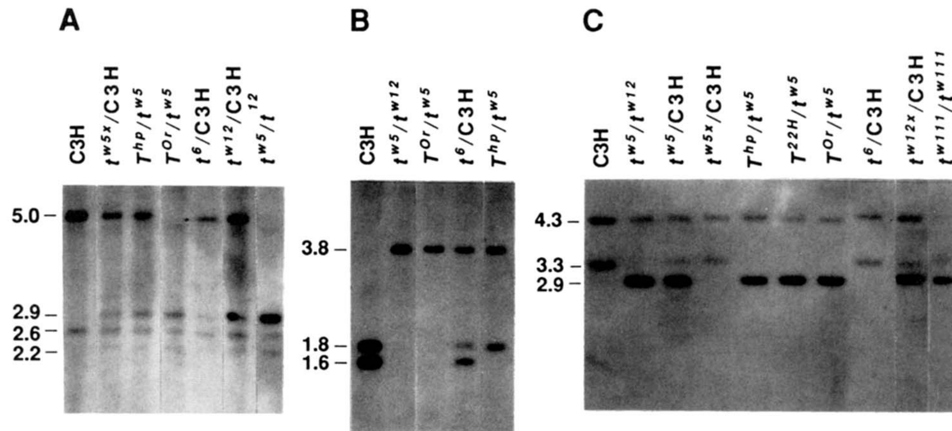

FIGURE 8.—Mapping of the *Au217* loci. (A) Hybridization of genomic probe P217a to *Hin*dIII-digested DNA. T^{Or} is deleted for both wild-type loci; since the 2.6-kbp wild-type is shared by *t*-haplotypes, only the 5.0-kbp band is missing. Similarly in T^{hp}, the 2.6-kbp fragment should be deleted. (B) Hybridization of genomic probe P217b to *Hin*dIII-digested DNA. Both wild-type fragments are deleted in T^{Or}, but only the smaller 1.6-kbp band is missing in T^{hp}. (C) Hybridization of P217c, a cDNA, to *Taq*I-digested DNA. Note that t^{w5x} is missing the amplified 2.9-kbp band while t^{w12x} retains it. Wild-type and *t*-haplotypes carry a 4.3-kbp fragment.

the flanking DNA from *Au217A* to a proximal position in *t*-haplotypes (*Au217rs*). In addition, judging from the band intensity seen on several different blots, the transcription unit seems to have been amplified severalfold. We have no evidence that the genomic DNA at the *Au217A* locus flanking the transcribed sequence also maps in this proximal position in *t*-haplotypes.

The P217c cDNA probe detects messages of ~2.4- and 3.0-kbp primarily in testis. (Trace amounts are seen in a few other adult tissues.) *Au217I* and *Au217II* may separately encode the two messages, but this has not been determined. The apparent higher copy number in *t*-haplotypes does not amplify message abundance. In fact, the opposite is true: Neither t^x/t^y nor T^{Or}/t testis RNA from C3H congenic animals contain any detectable message on northern blots (see Figure 5). It may be that the failure of *t*-haplotypes to transcribe this sequence is a result of genomic alterations that took place during the transposition and amplification events.

The proximal position of *Au217rs* in *t*-haplotypes allowed us to reexamine the t^{w12x} and t^{w111} haplotypes at this locus. In contrast to *Au116*, which exists in its wild-type form in these two exceptional recombinants, the P217c probe in both cases detects the 2.9-kbp *t*-type *Taq*I fragment (Figure 7C). This places the breakpoints of these two chromosomes between *Au116*/*Tu1* and *Au217rs*. Furthermore, since t^{w5x} has lost the 2.9-kbp *Taq*I fragment detected by P217c, we can order proximal end markers in *t*-haplotypes as: *cen* . . . (*Au116*/*Tu1*) . . . *Au217rs* . . . *Tctex-1* . . . (*Au9*/*Leh48*).

DISCUSSION

The five chromosome *17* markers described here are located in the interval between *Tu1* and *Leh66D*. This region encompasses *In(17)1* and *2* of *t*-haplotypes and is collectively known as the proximal end of the *t*-complex. *Au116* maps to the centromeric end of the *t*-complex near *Tu1*. *Au217I* and *II* are found in the inverted repeats in wild-type chromosomes and *Au217A* maps to the single copy of this region found in *t*-haplotypes. *t*-Haplotypes carry additional copies of the *Au217A* encoded transcription unit proximal to *Tctex-1*. *Au76* is located in the region deleted in t^{lub2}. Both *Au10* and *Au119* are near the *quaking* locus.

Recombination near the start of *In(17)1*: The serendipitous detection of three recombination events occurring in or near *In(17)1* has allowed the ordering in *t*-haplotypes of markers just distal to *Tu1* as *cen* . . . (*Tu1*/*Au116*) . . . *Au217rs* . . . *Tctex-1*. There may be a higher frequency of crossover in this region than normally seen between wild-type chromosomes and *t*-haplotypes. This raises two possibilities: Either the region around *Tu1*/*Au116* is outside of *In(17)1* and not under recombination suppression like the loci within the inversions or elements exist within *In(17)1* which can act as sites of exchange between *t*-haplotypes and wild-type chromatin. The latter case might be roughly analogous to the inverted repeats to which *Au217I* and *II* map which can generate rare crossovers between wild-type and *t*-haplotypes in *In(17)2* (HERRMANN, BARLOW and LEHRACH 1987).

The P217c probe shows that the t^{w5x} breakpoint in *t*-haplotypes occurred more distal to those of t^{w111} and t^{w12x}. It is possible that all three exchanges occurred proximal to *In(17)1* in *t*-haplotypes since the position of *Au217rs* with respect to the proximal breakpoint of *In(17)1* is not known. On the other hand, if *t*-chromatin (which is defined here as DNA under recombination supression whether it is within an inversion or adjacent to one) is being lost by recombination, it may be informative to assess the impact of this loss on the transmission ratio of *t*-haplotypes, as others

have proposed a locus, *Tcd-1*, at the proximal end of *t*-haplotypes which influences this phenotype (LYON 1984; SILVER and REMIS 1987).

If the proximal position of *Au217rs* in *t*-haplotypes is a consequence of a transposition-like event as we propose, we should not expect an allele at the corresponding site in wild-type chromosomes. However, DNA flanking the insertion site in *t*-haplotypes could yield a marker at this locus in wild-type chromosomes. The band intensity of the transposed *Au217rs* sequence indicates it is amplified in copy number, perhaps three- to fivefold. A similar event has been described by DISTECHE, GANDY and ADLER (1987). In this case, a 20-copy 9-kbp nonretroviral element in inbred mice was shown to have been translocated from the *X* chromosome centromeric region to an autosomal centromeric region where it is amplified about fivefold. These events occured independently in five seperate mouse strains, often on a different autosome. Also, UEHARA *et al.* (1990) identified an element (TSE, *t*-specific element) amplified to different extents in different *t*-haplotypes. Some *t*-haplotypes carried only a few copies while others carried over 100. In neither case, however, was a transcription unit identified.

The *Au217* locus is not expressed in *t*-haplotypes: The P217a and P217b genomic probes show that the *Au217* genomic DNA is duplicated in wild-type mice (loci: *Au217I* and *Au217II*), but is single copy in *t*-haplotypes (locus: *Au217A*). The mapping data are consistent with a position on the inverted repeats found in wild-type chromosomes (HERRMANN, BARLOW and LEHRACH 1987). We have verified this data by linking *Au217I/II* with *Leh66EI/EII* and *Leh119I/II* on pulsed-field gel fragments in wild-type mice (data not shown).

The *Au217I* and *II* loci encode two testicular cell transcripts in wild-type mice. (We have not determined if they are expressed from both loci or only one of the loci). These transcripts are not expressed from the *Au217A* locus in *t*-haplotypes or from the transposed and amplified copies in *t*-haplotypes. Hence, no transcript could be detected in testis RNA from t^x/t^y males or from T^{Or}/t and T^{22H}/t males since T^{Or} and T^{22H} delete both wild-type copies. Could these genes play a role in the sterility or transmission ratio distortion associated with *t*-haplotypes? These phenotypes are very likely the result of alterations in multiple factors acting in a concerted fashion (LYON 1984, 1986; SILVER and REMIS 1987). Because T^{Or}/t males are fertile (BENNETT and ARTZT 1990), loss of expression from *Au217A* alone is not sufficient to induce sterility; it may be that the loss of these transcripts in complete *t*-haplotypes acts in combination with mutations at other proximal or distal loci to produce sterility in t^x/t^y males. These sorts of interactions have been proposed, based on analysis of the t^6 partial distal *t*-haplotype and derivative chromosomes when heterozygous with a complete *t*-haplotype. The t^6 haplotype is a partial *t*-haplotype which carries wild-type chromatin proximal to the breakpoint between *Leh119I* and *Leh66EI* (HERRMANN, BARLOW and LEHRACH 1987). Since t^6 retains the 3.3-kbp wild-type band with the P217c probe (data not shown), *Au217I* should map proximal to this breakpoint. Evidence that t^6 has lost some of the *t*-genes involved in sterility has been presented by LYON (1986). It may be that the wild-type copy of the gene at *Au217I* (assuming that it expresses at least one of the two transcripts) is one of the loci involved.

Detection of a gene family expressed during development: The *Au76* locus contains one member of a small gene family that maps to the region deleted in the partial *t*-haplotype, t^{Lub2}. We have identified a sequence of at least 300-bp shared among the family members which is responsible for the observed cross-hybridization. *In situ* hybridization with the 1.4-kb cDNA indicates that message is present as early as 6.5 days p.c., and throughout embryogenesis. However, the signal becomes confined mainly to the CNS in late stages (O. RHO, unpublished data). This expression data does not distinguish among messages from different loci.

The *quaking* locus: Analysis of the region between *T* and *D17RP17* has suffered from a lack of markers. In particular, the *qk* locus, ~0.3 cM proximal to *RP17* is an interesting target for cloning. The genetics of the *qk* alleles available suggest the existence of one or more genes at this locus functioning in myelination of the central nervous system, spermiogenesis and embryogenesis. When homozygous, the original spontaneous mutation, qk^v, causes dysmyelination of the CNS, resulting in the quaking phenotype (SIDMAN, DICKIE and APPEL 1964), and complete arrest of spermiogenesis resulting in male sterility (BENNETT *et al.* 1971). There are five independent ENU-induced alleles of *qk* which are presumed to be point mutations. When these alleles are heterozygous with qk^v, quaking but fertile males are obtained. Homozygosity of the ENU-induced alleles results in embryonic lethality (JUSTICE and BODE 1988; KING and DOVE 1991).

Preliminary pulsed-field gel data indicate that the markers *Au10* and *Au119* can be physically linked to the *RP17* locus. Both are probably proximal to *RP17* in wild type. We have shown that at least 40-kbp at the *Au119* locus is deleted in qk^v homozygotes, while the neighboring locus, *Au10*, is not. Furthermore, the *RP17* locus is not deleted in qk^v/qk^v mice (data not shown). The size of the deletion has not yet been ascertained, however it is not large enough to significantly distort recombination frequency between *T* and *RP17* (KING and DOVE 1991). No transcripts in brain,

testis or embryo have been found encoded at *Au119* or *Au10* at the level of sensitivity of northern blots. It seems plausible that the male sterility associated with qk^v/qk^v, but not found with the ENU-induced mutations, could be due to a deleted gene necessary for spermiogenesis. However, the association of the quaking phenotype with a deletion presents a dilemma; since a presumed point mutation in the *qk* structural gene is an embryonic lethal, it is difficult to imagine that a null mutation resulting from a deletion would be homozygous viable. More likely, the deletion interferes with regulatory elements necessary in the CNS, but not with those needed during embryogenesis. Based on these assumptions, one could predict that the structural locus for *qk* should be found on one side or the other of the deletion breakpoint close to the junction site in qk^v/qk^v DNA.

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