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Mapping to molecular resolution in the *T* to *H-2* region of the mouse genome with a nested set of meiotic recombinants

(chromosome mapping/restriction fragment length polymorphism mapping/ethylnitrosourea/developmental mutants/quaking locus)

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ABSTRACT **We describe a meiotic fine-structure mapping strategy for achieving molecular access to developmental mutations in the mouse. The induction of lethal point mutations with the potent germ-line mutagen *N*-ethyl-*N*-nitrosourea has been reported. One lethal mutation of prime interest is an allele at the quaking locus on chromosome 17. To map this mutation, quaking$^{lethal\text{-}1}$, we have intercrossed hybrid mice that carry distinct alleles at many classical and DNA marker loci on proximal chromosome 17. From this cross we have obtained 337 animals recombinant in the *T* to *H-2* region. This number of crossovers provides a mapping resolution in the size range of single mammalian genes if recombinational hot spots are absent. DNA samples obtained from these recombinant animals can be used retrospectively to map any restriction fragment length polymorphism in the region. This set of DNA samples has been used to map the molecular marker *D17RP17* just distal of quaking$^{lethal\text{-}1}$. With the nested set of crossover DNA samples and appropriate cloning techniques, this tightly linked marker can be used to clone the quaking locus.**

Mouse developmental mutants are needed to identify genes essential for early mammalian development. Ideally, analysis of such mutants includes a molecular characterization of these genes, their products, and patterns of expression. Mouse mutants that carry single loss-of-function mutations on defined genetic backgrounds can facilitate the rigorous assignment of cloned molecules to developmental phenotypes by transgenic rescue (1). Furthermore, these mutants would permit the production of transgenic mouse strains in which the gene product of interest is systematically altered in structure or in pattern of expression.

Approaches to mouse developmental genetics that rely on spontaneous, radiation-induced, or insertional mutants have been largely successful (2, 3). However, the use of such variants can make the molecular characterization of affected genes and gene products difficult. For logistical reasons, most spontaneous and radiation-induced developmental mutants have been discovered on the basis of visible effects in heterozygotes. This emphasis on the subset of developmental mutant alleles with both dominant visible and recessive lethal phenotypes limits the classical approach to mouse developmental genetics. In addition, this obligatory coupling of dominant and recessive developmental effects often introduces mutational complexity. A dramatic case in point is the set of *t* haplotypes of the mouse, identified by their interaction with brachyury (*T*) to yield taillessness. In these variants, inversions link together multiple genes that affect embryonic viability, male fertility, and meiotic segregation ratios (4). Insertional mutations are, in theory, uniquely amenable to molecular analysis. Here, retroviruses or other cloned DNA elements are introduced into the germ line, and mutant lines with developmental lesions can be identified by inbreeding (3). The primary advantage of insertional mutagenesis is realized when the wild-type segment tagged by the insertion is cloned, reintroduced by transgenesis, and the homozygous mutant rescued. However, the specificity of tagging may be compromised when effects on expression extend quite far from the site of integration (5–7). Furthermore, the low rate of insertional mutagenesis (8) prohibits saturation of particular genetic regions or developmental processes with mutations (see ref. 9).

We have been exploring an alternative strategy for achieving the central goal of relating developmental lesions in mammals to particular gene products. Here, we rely on the very efficient point mutagenesis of the male germ line by *N*-ethyl-*N*-nitrosourea (EtNU), which can reach forward rates of $0.5–1.0 \times 10^{-3}$ per locus (10). With this agent, it is feasible to induce and screen for simple mutations on a fixed genetic background. Indeed, saturation of particular genomic regions with mutations is being approached (11–13), with multiple alleles being obtained as a by-product of saturation (14, 15). But how can we attain molecular access to loci defined only by point mutations?

We now report a fine-structure mapping analysis aimed at answering this question. The mutation quaking$^{lethal\text{-}1}$ ($qk^{l\text{-}1}$) is an EtNU-induced allele at the pleiotropic quaking (*qk*) locus on chromosome 17 (15). We have intercrossed hybrid mice that are heterozygous at many loci in the proximal region of chromosome 17, including *T*, *qk*, and the mouse major histocompatibility complex (*H-2*). From this cross we have recovered >300 progeny recombinant in the region that extends from *T* to *H-2* [≈15 centimorgans (cM) or 24,000 kilobase pairs (kbp) (4)].

MATERIALS AND METHODS

Mice. Animals were maintained and bred by standard methods of mouse husbandry (16). The BTBR strain, homozygous for tufted (*tf*) and segregating *T*, was inbred by J.-L. Guénet at the Institut Pasteur (Paris). All classical genetic loci are described by Green (17). The lethal quaking mutation, $qk^{l\text{-}1}$, was induced on the BTBR background with EtNU as described (12). T^{Wis}, a spontaneous brachyury allele, was discovered in the process of establishing a 129/Sv-$H\text{-}2^a$ congenic line. T^{Wis} confers a fully penetrant tailless phenotype. After 12 generations of backcrossing to strain 129/Sv ($H\text{-}2^{bv1}$), a T^{Wis}-$H\text{-}2^{a/bv1}$ male was bred to A/J females to yield T^{Wis} $H\text{-}2^a/+H\text{-}2^a$ animals, which were then maintained by backcrossing to A/J. T^{Wis} carriers from the first backcross to A/J were crossed to BTBR ($H\text{-}2^b$) mice that

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Abbreviations: EtNU, *N*-ethyl-*N*-nitrosourea; cM, centimorgan.

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carried qk^{l-1} balanced by *T*. The extremely short-tailed offspring, genotypically T^{Wis} + + $H\text{-}2^a$/+ qk^{l-1} *tf* $H\text{-}2^b$, were intercrossed and the progeny were scored visually for tail type and for H-2K serotype by complement-mediated cytotoxicity (18). Antisera used in the cytotoxicity assay were D-33 (anti-H-2K^b) and Y1-9-03-15-03 (anti-H-2K^k), obtained from the Transplantation Immunology Branch, National Institute of Allergy and Infectious Diseases (Bethesda, MD). Animals found to be recombinant were typed for H-2K at least once more to ensure accuracy. Recombinants were then progeny tested for qk^{l-1} and *tf* with mice carrying quaking^{viable} (qk^v) (19) and *tf*. The resulting progeny were scored at 3–5 wk for the transient quaking phenotype exhibited by animals genotypically qk^{l-1}/qk^v (15) and by weekly inspection extending to at least 8 wk for the tufted phenotype. Animals carrying the qk^v mutation were the gift of V. Bode (Kansas State University, Manhattan, KS).

Southern Blot Analysis. After having been scored for *H-2* and visible markers, animals were sacrificed, and the tissues were removed and frozen in liquid nitrogen. Livers were individually quick-frozen and then stored at −80°C as a back-up resource. High molecular weight DNA was isolated from frozen tissues by the method of Blin and Stafford (20). Approximately 10 μg of restriction endonuclease-digested DNA per lane was electrophoresed through 0.6% agarose and transferred to Zetaprobe (Bio-Rad) filters with 0.4 M sodium hydroxide, according to the manufacturer's instructions. Filters were prehybridized for 6–8 hr at 65°C in 0.25 M sodium phosphate, pH 7.2/5% $NaDodSO_4$/1 mM EDTA/10% (wt/vol) PEG 6000–8000/sonicated herring testis or calf thymus DNA (100 μg/ml). Hybridization probes were radiolabeled by nick-translation (21). Whole plasmids were as follows: pMK174 for locus *D17RP17* (*RP17*) (22) and p54-1.5 for locus *D17Leh54M* (*54M*) (23). Hybridizations were carried out at 65°C for 16–18 hr in fresh prehybridization solution with labeled probe added. Filters were washed to high stringency (final wash in 0.04 M sodium phosphate/1% $NaDodSO_4$ at 60°C) with gentle agitation. Washed filters were exposed to Kodak XAR-5 film with an intensifying screen at −70°C for at least 72 hr.

RESULTS

Screening of Animals Recombinant in the *T* to *H-2* Region. Hybrid mice carrying homologs for chromosome 17 that were derived from distinct genetic backgrounds were intercrossed. The heterozygous loci on proximal chromosome 17 included *T*, *qk*, *tf*, and *H-2*, as shown in Fig. 1. The brachyury allele used, T^{Wis}, confers an extreme and fully penetrant phenotype. The quaking allele, qk^{l-1}, is an EtNU-induced lethal mutation that complements the recessive sperm maturation defect of qk^v but only partially complements the neurological defect (15). In addition to these marker differences, the two homologs used carry alternative alleles at many DNA loci in this region (24, 25).

The phenotypes and genotypes of mice expected from this balanced lethal cross are shown in Punnett square format (Fig. 1). Although mice with the parental tail and H-2$^{a/b}$ phenotype (SH in Fig. 1) include some recombinants, these animals were discarded, with the exception of five *tf*/*tf* recombinants [genotypes *5* or *6*, marked (*) in Fig. 1]. All other phenotypic classes consist of informative recombinant animals for mapping qk^{l-1}, provided that double-recombinant and unequal-crossover genotypes are very rare or can be unambiguously detected. Among 1346 total progeny, we recovered 167 short-tailed, H-2$^{a/a}$ (SA); 134 short-tailed, H-2$^{b/b}$ (SB); 25 normal-tailed, H-2$^{a/b}$ (NH); and 6 normal-tailed, H-2$^{a/a}$ (NA) mice. The normal-tailed, H-2$^{b/b}$ (NB), class would be uniquely composed of double-recombinant gametes. Notably, no NB progeny were found.

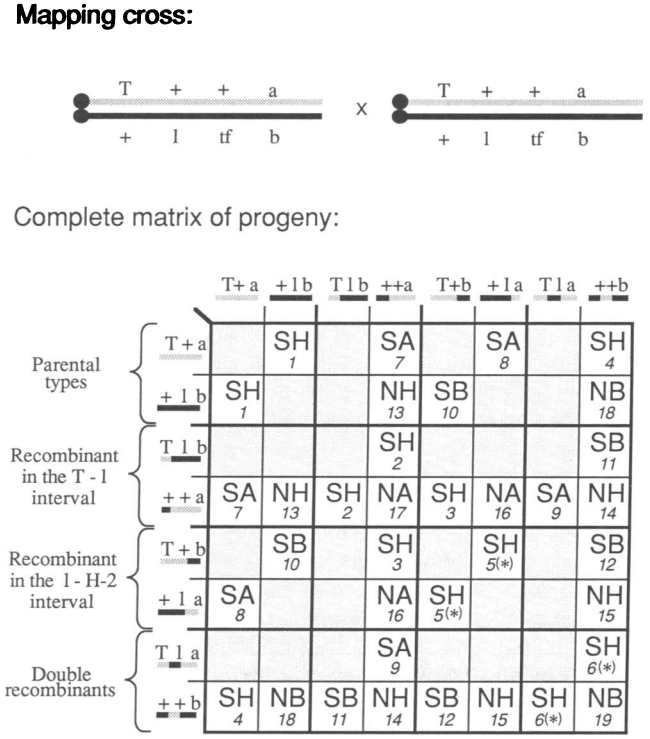

FIG. 1. Multiply heterozygous balanced lethal intercross and resulting progeny. Only chromosome 17 is drawn. Stippled bars, chromosome segments from the T^{Wis} line; solid bars, segments from BTBR. Centromeres are represented by knobs at the ends of the chromosomes. T, T^{Wis}; l, qk^{l-1}; a, $H\text{-}2^a$; b, $H\text{-}2^b$. Gametic genotypes are shown at the left and top sides of the matrix; only *T*, *qk*, and *H-2* loci are considered. Viable phenotypes are S, short tail; N, normal tail; H, H-2$^{a/b}$; A, H-2$^{a/a}$; B, H-2$^{b/b}$. Lethal gametic combinations are indicated by shading. Numbers in italics designate each particular genotype and are referred to in the text and in Table 1. (*) marks SH progeny that can be homozygous for *tf*.

A simple progeny test with normal-tailed qk^v homozygotes or carriers (male homozygotes are sterile) can identify carriers of qk^{l-1} and determine its phase (either cis or trans) with T^{Wis} (see *Materials and Methods*). In this way, the genotype of all recombinants can be verified, with the exception of genotypes *13* and *15* (see Fig. 1), which are not distinguishable on the basis of tail type and lethal status. The results of progeny testing are shown in Table 1. All 5 tufted SH mice were found to carry qk^{l-1} in trans with T^{Wis} (genotype *5*). Among 157 SA animals that were successfully bred, 23 did not carry qk^{l-1} (genotype *7*), while 134 carried qk^{l-1} in trans with T^{Wis} (genotype *8*). All 124 SB recombinants that were progeny tested carried qk^{l-1} in trans with T^{Wis} (genotype *10*). All 22 NH mice that gave progeny were found to segregate qk^{l-1} (genotype *13* or *15*). All 5 progeny-tested NA mice carried qk^{l-1} (genotype *16*).

Mapping T^{Wis}, qk^{l-1}, and *H-2K*. This three-point cross permits calculation of map distances for the regions between T^{Wis}, qk^{l-1}, and *H-2K*. Because these data are derived from intercrosses rather than backcrosses, they yield the average of the male and female recombination rates. If the frequency of double-recombinant genotypes approaches zero, then

$$\text{NH} + \text{SB} \approx \text{SA} \approx 2[p/2][1 - (p + d)/2] + 2[d/2][1 - (p + d)/2],$$

where p is the frequency of gametes recombinant in the proximal interval (between T^{Wis} and qk^{l-1}) and d is the frequency of gametes recombinant in the distal interval (be-

Table 1. Classification of progeny from the multiply heterozygous balanced lethal intercross

Tail type (number)	H-2 type (number)	T-l phase	Genotype designation	Total of each genotype scored
S (1517)	H (1009)		*1*	
			2	
			3	
			4	
			5	
			6	
	H (5)	T+/+l	*5**	5
		Tl /++	*6**	0
	A (167)	T+/++	*7*	23
		T+/+l	*8*	134
		Tl /++	*9*	0
	B (134)	T+/+l	*10*	124
		Tl /++	*11*	0
		T+/++	*12*	0
N (33)	H (25)	+l /++	*13* or *15*	22
		++/++	*14*	0
	A (6)	+l /++	*16*	5
		++/++	*17*	0
	B (0)		*18*	0
			19	0

Mice were scored for tail type (S, short; N, normal) at birth. H-2 typing was performed at least 1 wk after weaning (by which time some animals had died). H, $H\text{-}2^{a/b}$; A, $H\text{-}2^{a/a}$; B, $H\text{-}2^{b/b}$; T, T^{Wis}; l, $qk^{l\text{-}1}$. SH mice scored as nontufted were discarded. After confirming the H-2 serotype, recombinants were progeny tested for $qk^{l\text{-}1}$: possible genotypes are indicated in the column T-l phase. Genotype designation refers to genotypes illustrated and coded in Fig. 1. The far right column lists the total numbers of mice whose genotype was determined by progeny testing.
*SH progeny that were *tf* homozygotes.

tween $qk^{l\text{-}1}$ and *H-2K*) (see Fig. 1). In this limiting case, the frequency of gametes recombinant between T^{Wis} and *H-2K*, *f*, is $p + d$. Thus,

$$\begin{aligned} \text{NH} + \text{SB} + \text{SA} &= 4(p/2)(1 - f/2) + 4(d/2)(1 - f/2) \\ &= p - pf + d - df \\ &= p + d - f(p + d) \\ &= f - f^2. \end{aligned}$$

This expected frequency for NH plus SB plus SA classes is based not on the number of live-born mice (N) but on the total number of zygotes formed (N_z). From the Punnett square, N_z is given by $2N + p^2/2$. Because p is very small compared with N, $N_z \approx 2N$. Setting the actual frequency of NH, SB, and SA recombinants equal to the expected frequency,

$$326/(2 \times 1346) = f - f^2,$$

and solving the quadratic equation gives 14.1% ± 0.8% recombination (±1 SE) between T^{Wis} and *H-2K*.

In the same manner, the expected and observed frequencies of lethal-free SA (genotype *7* in Fig. 1) plus NH progeny indicate 2.1% ± 0.3% recombination between T^{Wis} and $qk^{l\text{-}1}$. On the basis of the frequency of SB and SA lethal carriers (genotype *8*), 12.0% ± 0.6% recombination separates $qk^{l\text{-}1}$ and *H-2K*.

Mapping the Visible Marker *tf*. All recombinants were also scored for the tufted phenotype. In addition, all nontufted recombinants were progeny tested to identify *tf* carriers. Among 270 crossovers between $qk^{l\text{-}1}$ and *H-2K*, 137 occurred distal of *tf*. Thus, the *tf* locus is placed 133/270 or 0.49 of the distance between $qk^{l\text{-}1}$ and *H-2K*.

Mapping the DNA Markers *RP17* and *54M*. The heterozygous parents used in this balanced lethal cross carry distinct homologs for chromosome 17 (24, 25). The $qk^{l\text{-}1}$-bearing chromosome 17, from BTBR, carries *b* alleles at DNA loci including *RP17* and *54M*. By contrast, the T^{Wis}-bearing chromosome 17 encodes *d* alleles at these loci (Fig. 2). These polymorphisms allow the scoring of recombinant progeny for the segregation of alternative alleles.

After progeny testing for $qk^{l\text{-}1}$ and *tf*, animals were sacrificed and tissues were taken for DNA isolation. This set of recombinants includes crossovers distributed as follows: 50 in the region from T^{Wis} to $qk^{l\text{-}1}$, 133 in the $qk^{l\text{-}1}$ to *tf* region, and 135 between *tf* and *H-2K* (see Fig. 3). Tissues from most

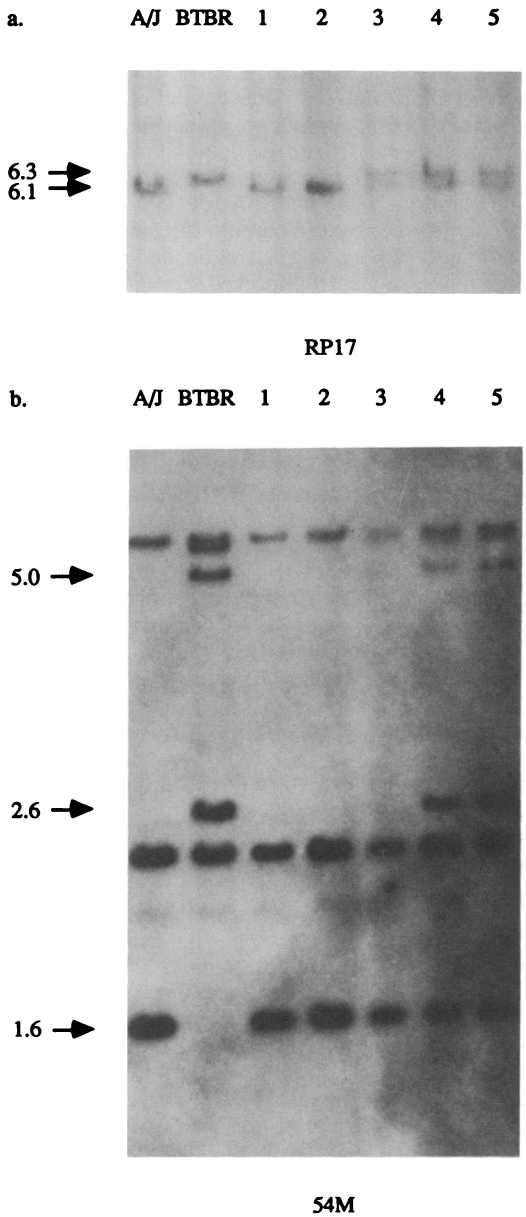

FIG. 2. DNA marker typing at *RP17* and *54M*. Restriction endonuclease-treated DNA from A/J, BTBR, and five recombinant animals (each with a crossover in a different marked interval) was electrophoresed and Southern blotted. Animal 1 is $T^{Wis} + H\text{-}2^a/+ + H\text{-}2^a$; animals 2, 3, and 4 are $T^{Wis} + H\text{-}2^a/+ qk^{l\text{-}1} H\text{-}2^a$; animal 5 is $T^{Wis} + H\text{-}2^b/+ qk^{l\text{-}1} H\text{-}2^b$. Among these recombinants, only animal 5 carries *tf*. (*a*) *Bam*HI-digested DNA, probed with pMK174 to reveal *b* (6.3 kbp) and/or *d* (6.1 kbp) alleles at *RP17*. (*b*) *Taq* I-digested DNA, probed with p54-1.5 to reveal *b* (5.0 and 2.6 kbp) and/or *d* (1.6 kbp) alleles at *54M*.

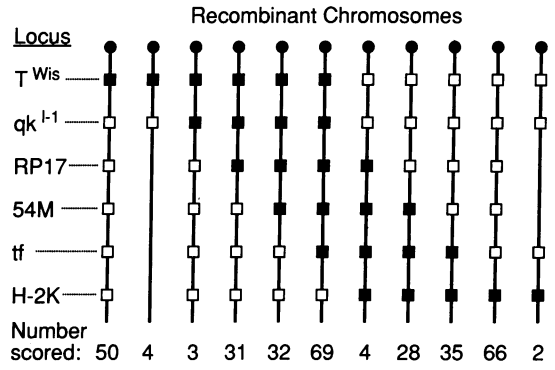

FIG. 3. The nested set of recombinant chromosomes. Squares represent loci that have been typed. ■, Alleles from BTBR; □, alleles from the T^{Wis} line. The total number of each type of crossover chromosome is shown below. Centromeres are represented by a knob at the top of each chromosome. Partially defined chromosomes represent recombinant animals that died before testing could be completed.

of the animals with a crossover in the region from *tf* to *H-2K* have been frozen and saved for future processing and analysis. From each of the other tissue samples, DNA was prepared and typed for *RP17* and *54M*. These two markers further subdivide the qk^{l-1} to *tf* region into three intervals. Southern blot analysis of DNA from five representative animals, each with a crossover in one of the intervals defined by all six markers, is shown in Fig. 2. A total of 7 crossovers were scored between qk^{l-1} and *RP17* (lanes 2), 59 between *RP17* and *54M* (lanes 3), and 67 between *54M* and *tf* (lanes 4). Fig. 3 summarizes the data from all samples for which the crossover in the T^{Wis} to *H-2K* region was assigned to a discrete interval. Fig. 4 displays the composite genetic map.

Search for Chromosomes Arising from Either Multiple Crossovers or Gene Conversions. Double crossovers and gene conversions would generate NB recombinants (see Fig. 1). No such animals were observed. In addition, animals with the double-recombinant genotypes labeled *6**, *9*, *11*, *12*, and *14* (see Fig. 1 and Table 1) would have been detected in crosses to qk^{v} carriers by the absence of qk^{l-1} or by its phase with respect to T^{Wis}. Of the 313 recombinant animals that were analyzed, none with any of these genotypes was observed. Furthermore, the segregation of *tf*, *RP17*, and *54M* alleles in all of the animals typed is consistent with single-crossover events. For example: no tested NH or SB animal is homozygous for alleles brought in by the T^{Wis} strain at any tested locus, and no SA animal is homozygous for BTBR alleles; all SA mice of genotype *7* are homozygous for alleles from the T^{Wis} strain at loci distal of *qk*, and all NH mice are heterozygous in the same region (see Fig. 3). However, 24 of 337 recombinants could not be progeny tested for qk^{l-1}. Also, the SH tufted phenotype could have resulted from either a

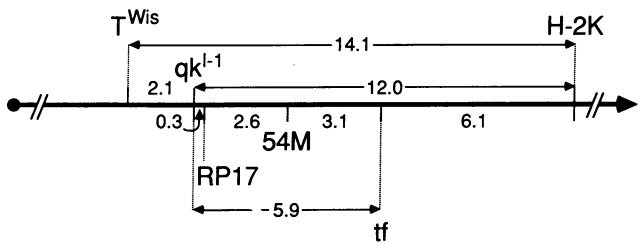

FIG. 4. Genetic map of chromosome 17. Genetic distances shown above the chromosome are percentage recombination directly measured as described in the text. Distances shown below the chromosome are based on the proportional dissection of each interval by the numbers of crossover chromosomes shown in Fig. 3.

double-recombinant genotype, T^{Wis} + *tf H-2*^{a}/+ qk^{l-1} *tf H-2*^{b}, or from the combination of two single-crossover gametes, T^{Wis} + *tf H-2*^{b} and qk^{l-1} *tf H-2*^{a}, as we have assumed in Table 1 and Fig. 3. The phase of T^{Wis} or qk^{l-1} with *H-2* alleles would distinguish these possibilities but was not tested.

Lack of Evidence for Unequal Crossing-Over. Unequal crossing-over can result in duplicated or deleted sequences in the region of the crossover (26). Such abnormalities may be revealed by analyzing the recombinant chromosome (detected in trans with the T^{Wis}-*H-2*^{a}-chromosome 17) in trans with a BTBR chromosome. Accordingly, eight SA animals with crossovers either between T^{Wis} and qk^{l-1} or between qk^{l-1} and *tf* were bred with normal-tailed BTBR mice. DNA was isolated from normal-tailed progeny and scored for loss or duplication of *RP17* or *54M*. Because rare new crossovers arising during meiosis in the SA parent could yield misleading results, at least two progeny from each cross were examined. This preliminary survey failed to detect unequal crossovers in this region (data not shown).

DISCUSSION

We report a meiotic fine-structure mapping strategy for linking the genetic map of mutationally defined developmental loci to the corresponding molecular organization. The DNA samples saved from this large set of meiotic recombinants can be used to map any polymorphic marker on proximal chromosome 17 to high resolution. This process would identify a tightly linked DNA marker that can be used to select wild-type sequences that span the *qk* locus.

The mapping strategy described here depends on polymorphisms between inbred mouse strains derived from *Mus musculus*. The power of this analysis will be limited by the incidence of DNA polymorphism between the two homologs. For nonpolymorphic DNA clones, a linked polymorphism can be found by isolating a cosmid clone (27). Interspecific mapping crosses involving *Mus spretus* or *M. musculus castaneus* are of value in that most sequences are polymorphic in Southern blot analysis (28–30). However, the genetic distances derived from inter- and intraspecific crosses may be skewed. Indeed, an inversion involving the *M. spretus* chromosome 17 has been reported (29), as well as recombinational hot spots in crosses involving *M. musculus castaneus* (31, 32). Thus, data from interspecific crosses may not be informative in all parts of the genome, especially for high-resolution mapping.

DNA from animals that carry crossover chromosomes has been saved for further analysis as additional DNA marker probes become available. Unlike the familial panels of human DNA available from the Centre d'Etude du Polymorphisme Humaine (Paris) for the mapping of polymorphic loci in humans, this set of mouse DNA is immune to mutational drift that may arise in tissue culture. Also in contrast, DNA samples from this set of recombinant mice are recovered in the range of 10 mg per animal. While not infinite, this is adequate for at least 1000 Southern blot transfers. If nylon filters are stripped and reprobed successively, in the range of 10^4 Southern blot assays can be performed. Thus, DNA from these recombinants is probably sufficient in amount for the complete retrospective mapping analysis of the discrete region from T^{Wis} to *H-2K*.

The facility with which the presence of qk^{l-1} can be verified by progeny testing (see *Materials and Methods*) has permitted a full analysis of many of the recombinant genotypes arising in this study. The recombinant animals we have recovered are predominantly, if not exclusively, carriers of single-crossover chromosomes. If there were no interference over this 15 cM region, then 4 double-crossover chromosomes would have been expected. This suggestive evidence

for positive interference is consistent with more extensive evidence for strong positive interference over map intervals up to 15 cM in *Drosophila melanogaster* (33) and most fungi (34). Evidence for moderate positive interference over even larger distances is reported on the mouse X chromosome (30). The apparent absence of multiple and unequal crossover events revealed by this mapping analysis of the *T* to *H-2* region makes this a nested set of recombinant chromosomes wherein the lethal mutation qk^{l-1} can presumably be unambiguously ordered against each local polymorphic marker with which it recombines. To map other recessive lethals in the *T* to *H-2* region, recombinant sets specific to each must be made. In contrast to qk^{l-1}, these other lethal mutations are not known to interact with any chromosome 17 marker to yield a visible phenotype. Therefore, it will be difficult to progeny test for the respective lethal mutation. Positive interference in this region would generate a predominance of single-crossover chromosomes in all these sets.

The meiotic technique described can map a mutation into an interval defined by the nearest proximal and distal crossover points in the set of recombinants. The size of the mapping interval decreases in proportion to the number of recombinants obtained. However, mapping resolution could be limited by recombinational hot spots. We have found crossovers in every interval tested (Fig. 3), and the order and observed genetic distances between these markers (see Fig. 4) are comparable to those reported elsewhere (17). Therefore, no hot spots specific to this cross are apparent. A quantitative assessment of recombinational hot spots in the T^{Wis} to *H-2K* region awaits information on the physical distances between these markers. In the absence of hot spots, the average interval in the set of recombinants we report would be 24,000 kbp per 318 crossovers, or ≈75 kbp per crossover. The average spacing between transcribed regions in the mouse genome has been estimated to be between 35 and 75 kbp (31, 35–37). Therefore, the resolution reached with this nested set of recombinants approaches the range necessary to assign a point mutation or DNA marker to an interval the size of a single gene.

The degree of genetic resolution also corresponds to physical distances that overlap the size of DNA fragments that can be cloned as yeast artificial chromosomes (38). The panel of DNA described here will permit facile assignment of DNA clones to recombinant intervals tightly linked to the site of the lethal point mutation, qk^{l-1}. Once identified, such very close DNA markers can in turn permit the isolation of a wild-type DNA fragment capable of rescuing lethal homozygotes by transgenesis.

This meiotic mapping approach may be applied more generally in the mouse. Existing or induced mutations throughout the genome can be mapped to molecular resolution, provided that easily scored flanking markers and distinct but homologous chromosomes are available. The most efficient mapping cross (intercross or backcross) depends on the nature of the mutation of interest and the flanking markers used. Thus, the combination of efficient mutagenesis with high-resolution meiotic mapping can allow the molecular genetic analysis of developmental functions encoded throughout the mouse genome.

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