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Genetics

Mitotic recombination in germ cells generated two major histocompatibility complex mutant genes shown to be identical by RNA sequence analysis: K^{bm9} and K^{bm6}

(gene conversion/*Q4* gene/murine histocompatibility antigens)

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ABSTRACT RNA sequencing represents a major procedural simplification for nucleotide sequence analysis of a transcribed gene. Using newly adapted mRNA and cDNA sequencing procedures, we have sequenced 855 nucleotides of K^{bm9} mRNA, corresponding to the codons for the amino-terminal 285 amino acids. The inferred DNA sequence of the K^{bm9} gene differs from the parental K^b sequence by single nucleotide alterations in each of codons 116 and 121, resulting in Tyr→Phe and Cys→Arg substitutions, respectively. The K^{bm9} sequence is identical to that of another independently arising MHC mutant gene, K^{bm6}. As both the K^{bm9} and K^{bm6} genes were generated by recombination between the K^b and *Q4* genes, our data indicate that the identical genetic interactions have occurred at least twice. The relatively large extent of identity between *Q4* and K^b may be responsible for frequent recombination between the two genes. The parents of the original bm9 mutant mice had five identical mutant offspring, which can be explained by mitotic recombination in the germ cells, producing gonadal mosaicism in the C57BL/6 mother. Thus, mitotic recombination, and not meiotic recombination, appears to be responsible for the formation of at least some of the K^b mutants. Such a mechanism probably plays a major role in the generation of diversity in the major histocompatibility complex.

Class I genes of the major histocompatibility complex (MHC) constitute a multigene family consisting of loci for the classical transplantation antigens (*H-2*) and the *Qa-* and *Tla*-related molecules. Alleles of each of the *H-2* loci (*K*, *D*, and *L*) exhibit substantial structural differences (1–3). This diversity is accompanied by extensive polymorphism of these genes on the population level (4). In contrast, only a few alleles of the *Qa-* and *Tla*-region genes have been detected, and these alleles exhibit only limited diversity (2, 5–7). The biological and molecular processes that conserve *Qa* and *Tla* genes and diversify *H-2* genes are central to our understanding of the dynamic evolution of the MHC.

The spontaneous H-2K^b mutants, detected by skin-graft incompatibility among individuals of the parental C57BL/6 (H-2^b) strain, have provided a model system for studying the mechanisms responsible for the ongoing evolution of *H-2* genes, as such mutants represent a first step in the rediversification of the K^b gene (8–14). When compared to the parental molecule, the mutant K^b molecules exhibit a number of important characteristics (10, 15): clustered, multiple amino acid substitutions; multiple nucleotide alterations per codon; the occurrence of the same amino acid substitutions in independently arising mutants; and the presence of the substituted amino acids, at homologous positions, in other class I gene products. These characteristics have suggested that the K^b mutants are generated by recombination (also referred to in the literature as genetic interaction or gene conversion) between the K^b gene and other class I genes, rather than by classical, spontaneous point mutations (8–10). Evidence supporting this theory has accumulated with the cloning and sequencing of the K^{bm1} and K^{bm6} genes and the identification of class I genes in the parental genome that can donate nucleotide sequences identical to those substituted into the mutant genes (11, 13, 14, 16).

The "bg series" of mutants (bm5, bm6, bm7, bm9, bm16, bm17, bm18, bm20) is an extreme example of the independent recurrence of the same or similar mutations ("bg series" is an historical term for the members of this group of mutants, which were originally designated bg-1, bg-2, bg-3, etc.). All members of the "bg series" are histocompatible with one another but reciprocally incompatible with parental B6 (17). Peptide mapping and partial amino acid sequencing studies have indicated that the K^{bm5}, K^{bm6}, K^{bm7}, K^{bm9}, and K^{bm16} molecules share a Tyr→Phe substitution at amino acid position 116 (18, 19). In addition, the K^{bm6}, K^{bm7}, and K^{bm9} molecules have a Cys→Arg substitution at position 121 (19). The sequencing of the K^{bm6} gene has demonstrated that K^b and K^{bm6} are identical to one another except for single nucleotide substitutions in the codons for amino acids 116 and 121 (13). The *Q4* gene, located in the *Qa* region of the MHC, has been identified as the donor gene in the recombination event that generated the K^{bm6} mutation (13).

Analysis of *K* genes from additional members of the "bg series" is necessary to delineate the molecular events involved in the recombination between *K* genes and other class I genes. Such sequence analysis would determine whether mutants, identical by amino acid substitutions, are identical to one another on the nucleotide level. This information would indicate the number of genes capable of interacting with the K^b gene, as well as the frequency and extent of such interactions.

We describe two newly adapted, oligonucleotide-primed, dideoxynucleotide RNA and cDNA sequencing techniques that allow the sequencing of a mRNA transcript without prior cloning of the gene or purification of the specific mRNA. Using RNA and cDNA sequencing techniques, we have determined that the coding sequence of K^{bm9} differs from that of K^b by single nucleotide changes in each of the codons of amino acids 116 and 121. Thus, K^{bm9} and K^{bm6} are identical. Although the bm9 mutant arose in a (B6 × BALB/c)F_1, the K^{bm9} mutations did not result from recombination in the zygote between K^b and a gene of the H-2^d haplotype. Genealogical analysis indicates that the bm9 mutant resulted

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Abbreviation: MHC, major histocompatibility complex.
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from the mitotic recombination of K^b and *Q4* in the germ cells of the C57BL/6 mother.

MATERIALS AND METHODS

Mice. C57BL/6 (B6) and B6.C-H-2^{bm9} (bm9) mice were obtained from the breeding and screening laboratories of R.W.M. (Northwestern University Medical School, Chicago), C. David (Mayo Clinic, Rochester, MN), and The Jackson Laboratories.

Preparation of DNA, RNA, and Synthetic Oligonucleotides. The preparation of genomic DNA, polyadenylylated liver RNA, and synthetic oligonucleotides, as well as the methods of DNA size-fractionation in agarose gels, transfer to GeneScreen (New England Nuclear), hybridization, radiolabeling of oligonucleotides, and restriction endonuclease digestion, have been described (13). The bm6-specific oligomer is a 21-nucleotide-long probe complementary to the K^{bm6} sequence 5' AGTTCGCCTACGACGGCCGCG 3', corresponding to the codons for amino acids 115–122. It differs from the K^b sequence at nucleotide positions 4 and 18 (13).

Preparation and Radiolabeling of Oligomers for RNA and cDNA Sequencing. K^b-specific oligonucleotide primers (18–21 nucleotides long) were synthesized. A 0.1-μg sample of oligomer was radiolabeled in the presence of 50 mM Tris Cl (pH 7.6), 10 mM $MgCl_2$, 5 mM dithiothreitol, 0.1 mM spermidine, 100 μCi of [γ-^{32}P]ATP (Amersham; 5000 Ci/mmol; 1 Ci = 37 GBq) and 7 units of polynucleotide kinase (New England Biolabs; Bethesda Research Laboratories; IBI Technologies, New Haven, CT) for 30 min at 37°C.

RNA Sequencing Techniques. Previously described procedures for oligonucleotide-directed cDNA synthesis (20, 21) and dideoxynucleotide sequencing of immunoglobulin mRNA (22) were modified and incorporated to develop a protocol amenable to K^b-specific mRNA sequencing. Two procedures for the direct sequencing of RNA were devised. The identity of each nucleotide was determined at least three times by either RNA or cDNA sequencing, or both.

Procedure 1. ^{32}P-labeled oligonucleotide primer (10 ng) and poly(A)$^+$ RNA (2 μg) were heated at 80°C for 3 min in 15 μl of annealing buffer (250 mM KCl/10 mM Tris Cl, pH 8.3/1 mM EDTA) and allowed to anneal for 1 hr at a temperature dependent on the sequence of the oligonucleotide, determined by the formula T (°C) = 4(G + C) + 2(A + T) − 5 (see ref. 23). The RNA-primer annealing solution was added to 25 μl of transcription buffer [24 mM Tris Cl, pH 8.3/16 mM $MgCl_2$/8 mM dithiothreitol/0.4 mM dATP/0.4 mM dCTP/0.8 mM dGTP/0.4 mM dTTP/actinomycin D (100 μg/ml)], containing 3–6 units of avian myeloblastosis virus reverse transcriptase (Life Sciences, St. Petersburg, FL) and one dideoxynucleoside triphosphate (0.36 mM ddATP, 0.02 mM ddCTP, 0.02 mM ddGTP, or 0.56 mM ddTTP), and incubated at 50°C for 1 hr. The reaction was stopped by addition of EDTA (to 50 mM) and ammonium acetate (to 0.4 M) and ethanol precipitation. The ethanol precipitate was washed twice with 70% ethanol, dried, resuspended in loading buffer (100% formamide plus 0.3% bromophenol blue and 0.3% xylene cyanol FF), boiled for 3 min, and loaded onto a sequencing gel.

Procedure 2. ^{32}P-labeled oligonucleotide primer (5 ng) and poly(A)$^+$ RNA (10 μg) were heated at 80°C for 3 min in 12 μl of annealing buffer (see above) and allowed to anneal for 1 hr. Two microliters of the RNA-primer annealing solution were added to 3.3 μl of transcription buffer (see above), containing 3–6 units of reverse transcriptase and one dideoxynucleoside triphosphate (final concentration 0.15 mM ddATP, 0.15 mM ddCTP, 0.15 mM ddGTP, 0.30 mM ddTTP), and incubated at 50°C for 1 hr. The reaction was stopped by addition of 2 μl of loading buffer (see above). After the sample was boiled for 3 min, half (4 μl) was loaded onto a sequencing gel.

cDNA Sequencing Technique. cDNA generated by the primer-extension of 10–12 μg of poly(A)$^+$ RNA by procedure 1 was extracted sequentially with equal volumes of phenol, phenol/chloroform (1:1), and chloroform and then ethanol-precipitated 3 times. The dried pellet was resuspended in 10 μl of cDNA annealing buffer (10 mM Tris Cl, pH 7.5/5 mM NaCl) with 5 ng of ^{32}P-labeled oligonucleotide primer, boiled for 5 min, and allowed to anneal for 1 hr at 45–50°C. A 2-μl sample of the cDNA-primer annealing solution was added to 3 μl of polymerase buffer (as formulated by Pharmacia P-L Biochemicals, except dATP was added to 1.66 μM in the A reaction and to 33 μM in the C, G, and T reactions and the ddNTP concentrations were adjusted as follows: 0.7 mM ddATP, 0.26 mM ddCTP, 0.26 mM ddGTP, and 2.1 mM ddTTP) and 1 unit of Klenow polymerase (Bethesda Research Laboratories) and incubated at 42°C for 30 min. The reaction was stopped by addition of 2 μl of loading buffer and boiling for 3 min; half (4 μl) of the sample was loaded onto a sequencing gel.

RESULTS

Oligonucleotide-Primed, Dideoxynucleotide RNA and cDNA Sequencing Techniques. Dideoxynucleotide sequencing of abundant or purified RNA has been described (22, 24–28). We have successfully adapted these techniques for the sequencing of unpurified K^b mRNA, in spite of the relatively low frequency of H-2 mRNA [0.01–0.05% of poly(A)$^+$ RNA (20)]. K^b-specific oligonucleotides were synthesized, radiolabeled, and used as primers for sequencing K^b mRNA. The effectiveness of this approach is demonstrated in Fig. 1*a*, an autoradiogram of a portion of a bm9 RNA sequencing gel. In general, more than 250 nucleotides can be sequenced with a single ^{32}P-labeled primer, depending on the concentration of ddNTPs and the length of the gel. Further, the diversity of class I mRNAs is such that a locus-specific oligonucleotide primer can be constructed every 50–100 nucleotides, allowing for the sequencing of the entire coding portion of the K^b mRNA.

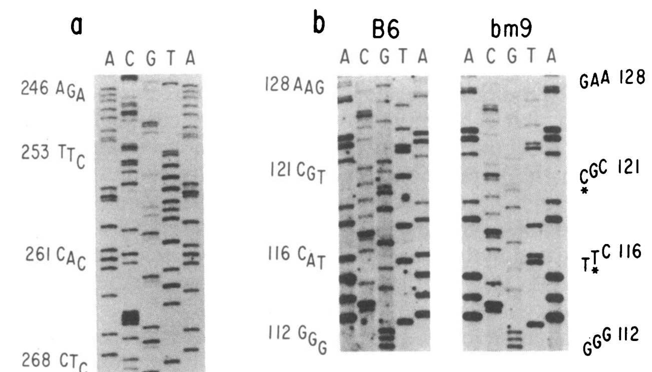

FIG. 1. Autoradiograms of RNA and cDNA sequencing gels. (*a*) RNA sequencing of K^{bm9}. Polyadenylylated bm9 RNA was sequenced as described in *Materials and Methods*. Sequencing was initiated with a K^b-specific oligonucleotide primer, complementary to the mRNA at codons for amino acids 294–301. The sequence, when read bottom to top, is complementary to the sense strand and in the 3'→5' orientation. Sequences shown correspond to codons 269–245 (bottom to top). (*b*) cDNA sequencing of K^{bm9}. cDNA was synthesized from polyadenylylated B6 and bm9 RNA and sequenced as described in *Materials and Methods*. Sequencing was initiated with a K^b-specific oligonucleotide primer, complementary to the cDNA codons for amino acids 94–100. The sequence, when read bottom to top, is the sense strand in the 5'→3' orientation. Sequences shown correspond to codons 112–128. Altered nucleotides in codons 116 and 121 of bm9 are underscored with asterisks.

Two artifacts often associated with dideoxynucleotide DNA sequencing—namely, premature termination and band compression—are also evident with the sequencing of RNA. The premature termination of a cDNA transcript results in the appearance of a band in all sequencing lanes. The selection of ddNTP concentrations that result in specific termination bands more prominent than "background" signals obviates this problem. Both artifacts, which affect the reading of several of the nucleotide positions of the K^b mRNA, can be virtually eliminated by dideoxynucleotide sequencing of uncloned cDNA generated in a primer-extension reaction (Fig. 1*b*). In this procedure, K^b mRNA is transcribed with reverse transcriptase, using a nonradioactive oligonucleotide primer. The cDNA generated in this reaction is then sequenced using a radiolabeled oligonucleotide primer, ddNTPs, and Klenow polymerase. The rationale behind this approach is that the sequencing of cDNA involves a different, complementary template (cDNA vs. RNA), a different enzyme (Klenow polymerase vs. reverse transcriptase), and a different buffer system. Thus, the possibility of an artifact affecting the same nucleotide position in both techniques would be very small. It is therefore possible, in effect, to sequence K^b mRNA in "both directions."

RNA and cDNA Sequence Analysis of K^{bm9}. Eight-hundred fifty-five nucleotides of K^{bm9} mRNA and cDNA were sequenced, corresponding to amino acids 1–285. The coding sequence of K^{bm9} is identical to that of K^b with the exceptions of single nucleotide changes in the codons for amino acids 116 and 121 (Fig. 1*b*). K^b and K^{bm9} differ by an A→T and a T→C substitution, resulting in Tyr→Phe and Cys→Arg replacements in amino acid positions 116 and 121 of K^{bm9}, respectively (Fig. 1*b*) These substitutions are identical to those reported for the K^{bm6} mutation (13) (Fig. 2). The nucleotide sequence of the third exon (α2 domain) of K^{bm9} is shown in Fig. 2 in comparison with K^b (29) and K^{bm6} (13).

Oligonucleotide Hybridization Analysis of B6, bm9, and bm6 DNA. The accuracy of the RNA and cDNA sequencing procedures can be confirmed by oligonucleotide hybridization studies. Since the K^{bm9} and K^{bm6} genes have the same nucleotide sequence, K^{bm9} would be expected to hybridize to the bm6-specific oligonucleotide probe, previously shown to hybridize to K^{bm6} but not to K^b (13). Fig. 3 shows that the probe hybridizes to 4.8-kilobase *Bam*HI fragments corresponding to the K^{bm9} and K^{bm6} genes, but not to the K^b gene, confirming the RNA and cDNA sequence data. The probe also hybridizes to a single donor sequence located on an 8.6-kilobase *Bam*HI fragment, previously shown to be the *Q4* gene, the only possible donor gene for the K^{bm6} mutation (13).

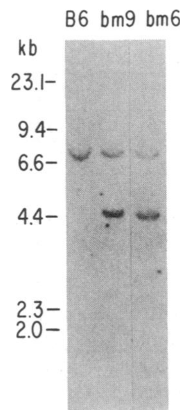

FIG. 3. Hybridization analysis of genomic DNA with the bm6-specific probe. Splenic DNA samples from B6, bm9, and bm6 mice were digested with *Bam*HI, size-fractionated by agarose gel electrophoresis, transferred to GeneScreen and hybridized to ^{32}P-labeled probe at 65°C for 16 hr. After hybridization, the filter was washed and exposed to Kodak XAR film for 2 days. Positions and sizes [in kilobases (kb)] of markers (*Hin*dIII-digested phage λ DNA) are at left.

These data now identify the *Q4* gene, located in the *Qa* region of the MHC, as the donor gene for both the K^{bm9} and K^{bm6} mutations. The substituted nucleotides in K^{bm9} are situated in a region where K^b and *Q4* are otherwise identical for 95 nucleotides, delineating the maximal genetic transfer between the two genes (Fig. 2).

Genealogical Analysis of bm Mutants Indicates Mitotic Recombination. The unequivocal designation of the *Q4* gene as the donor gene for the K^{bm6} mutation was based on the facts that the bm6 mutant arose from a homozygous B6 × B6 (H-2^b × H-2^b) mating and that *Q4* is the only gene in the B6 genome to contain the appropriate donor sequence (13). Since the bm9 mutant arose from a B6 × BALB/c (H-2^b × H-2^d) mating (17), the possibility exists that genetic interaction occurred in the zygote between the K^b gene and a donor gene of the H-2^d haplotype. The same point has also been raised concerning the generation of the bm1 mutant (11).

A review of the genealogical data (Table 1) indicates that both the bm9 and bm6 mutants appeared as multiple mutants within their sibships. Such data can best be interpreted by mitotic recombination. It is statistically unlikely that the five original bm9 mutants were the result of five identical zygotic recombination events. Further, five identical mutant progeny could not be derived from a single meiotic recombination event, as only one egg (and three polar bodies) is produced from an oocyte undergoing meiosis, and at most two recombinant sperm (and two parental-type sperm) are produced from a primary spermatocyte. In addition, the possibility of monozygotic quintuplets (bm9) and triplets (bm6) can be eliminated as mutants of both sexes were detected. However, if recombination occurred early in the mitotic amplification of the germ line, a sizable portion of the germ cells would carry

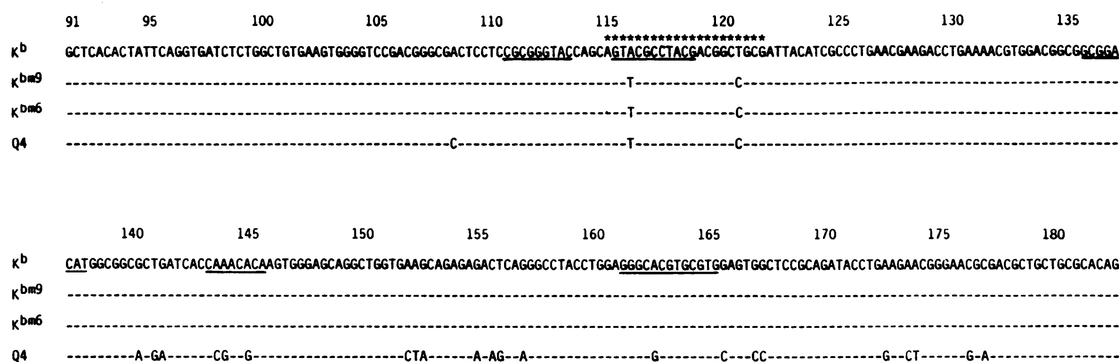

FIG. 2. Sequence comparison of the third exons (α2 domains) of K^b, K^{bm9}, K^{bm6}, and *Q4*. Numbers indicate the position of the amino acids coded for by the corresponding codons (according to refs. 1 and 29). Dashes in the K^{bm9}, K^{bm6}, and *Q4* sequences indicate identity to K^b. Two nucleotide differences exist between K^b and both K^{bm9} and K^{bm6}, at codons 116 and 121. Underlined sequences may have the potential to form Z-DNA under the appropriate conditions. Asterisks indicate where the bm6-specific probe would bind to K^{bm6}, K^{bm9}, and *Q4*. (K^b sequence from refs. 14 and 29; K^{bm6} and *Q4* sequence from ref. 13).

Table 1. Genealogical analysis of bm9 and bm6 mutant mice

Mutant	Mother*	Father*	Progeny	
			Normal	Mutant
bm9	C57BL/6Kh	BALB/cKh	14	5 (1♀/4♂)
bm6	C57BL/6Kh	C57BL/6Kh	11	3 (2♀/1♂)

Data summarized from ref. 17.
*Graft tested and found normal prior to mating. For explanation of graft testing and husbandry, see ref. 17.

the mutant gene. This would be consistent with the multiplicity of mutant mice initially detected. Thus, the bm9 mutation must have preexisted in the germ line of the B6 mother of the mutants and must have resulted from the genetic interaction of K^b and *Q4*. In addition, the original bm23 mutation was observed in four siblings (31). Therefore, at least 3 bm mutants (bm9, bm6, and bm23) appear to have been generated by mitotic recombination events during the amplification of the germ line.

DISCUSSION

RNA sequencing represents a major procedural simplification for nucleotide sequence analysis. This technique allows the sequencing of a transcribed gene without prior cloning, screening, and subcloning of the gene or its cDNA. Previously described dideoxynucleotide RNA sequencing techniques have utilized abundant or purified RNA such as immunoglobulin or viral RNA (22, 24–28). Less abundant mRNAs such as MHC class I mRNAs appear not to be amenable to dideoxynucleotide sequencing which incorporates [α-^{32}P]dNTP. However, by using radiolabeled, K^b-specific oligonucleotides as primers, we have successfully adapted the dideoxynucleotide procedure for the sequencing of unpurified K^b mRNA and uncloned cDNA. This procedure is particularly well suited for the study of parent–mutant combinations, as nucleotide alterations between the two are easily detectable in samples placed side-by-side in sequencing gel lanes.

We have also described a procedure whereby nucleotide sequences obtained by the RNA sequencing technique may be confirmed by "second-strand" sequencing of uncloned cDNA, previously generated in a primer-extension reaction. Nucleotide sequences obtained by this procedure are virtually free from sequencing artifacts. This procedure may prove to be an even more powerful technique than direct RNA sequencing. Once sequence differences are observed, oligonucleotide probes, complementary to mutant sequences, can be hybridized to parental and mutant genomic DNA. The hybridization of the oligonucleotide probe to mutant, but not parental, genomic DNA serves as a confirmation of nucleotide sequences obtained by RNA and cDNA sequencing techniques.

Using the above techniques, we have determined the nucleotide sequence of K^{bm9} mRNA. The K^{bm9} mutant was chosen for this study to determine whether K^{bm9} and K^{bm6}, which contain the same amino acid substitutions as determined by protein sequencing techniques (19), are also identical on the nucleotide level. Different nucleotide sequences among members of the "bg series" could result from the recombination of K^b with different donor genes. Our results show that K^{bm9} and K^{bm6} are identical on the nucleotide level, and indicate that the same mutation, involving the same donor gene, *Q4*, has occurred at least twice. Further, the finding that the K^{bm5} mutant is identically altered at amino acid position 116 suggests that this mutant is also the result of a recombination event between K^b and *Q4* (unpublished data). Thus, it is possible that *Q4* is the donor gene for the eight related recombination events that generated the "bg-series" mutants.

The extent of nucleotide identity between K^b and *Q4* that flanks the region of substitution in the K^{bm9} and K^{bm6} genes defines the maximal length of the recombination event (95 nucleotides between the codons for amino acids 108 and 140) (Fig. 2). This stretch of identity is larger than that observed between the genes interacting to generate the bm1 mutant (K^b and *Q10*, 50 nucleotides) (16) and the class II bm12 mutant ($I\text{-}A^b_\beta$ and $I\text{-}E^b_\beta$, 44 nucleotides) (30, 32). Upstream of a silent base mismatch in the codon for amino acid 108, K^b and *Q4* share an additional 64 nucleotides of identity (Fig. 2) (13). The relatively large stretch of identity between the K^b and *Q4* genes may be responsible for increased initiation of recombination between the two genes and the high frequency of bg series mutants. This idea is supported by data, obtained from prokaryotes, that indicate there is an exponential increase in the frequency of recombination when the length of identical DNA between two duplexes is increased from 20 to 73 nucleotides (33). The spatial orientation of K^b and *Q4*, as well as the nature of the changes involved, may also play a role in the recurrent recombinations resulting in bg-series mutations.

The recombination events between K^b and *Q4* that produced the identical K^{bm9} and K^{bm6} mutants did not extend beyond the codons for amino acids 108 and 140 (Fig. 2). This suggests that something intrinsic to the involved sequences modulates the initiation and/or termination of the short recombination events that are characteristic of the K^b mutants. The nucleotide sequences near the 5′ and 3′ termini of the K^b/*Q4* region of identity (i.e., codons for amino acids 111–115 and 136–138), consist of alternating purines and pyrimidines, with at most one nucleotide out of phase (Fig. 2, underlined sequences). Similar sequences appear at the termini of the K^b/*Q10* region of identity flanking the K^{bm1} substitutions (codons for amino acids 143–146 and 162–165) (Fig. 2). If these sequences were capable of switching into the Z-DNA conformation under the appropriate conditions of supercoiling, such an alternative DNA form could modulate the initiation and termination of recombination (34). Single-stranded DNA near the B/Z-DNA junction could be nicked by nucleases and result in the displacement of one strand of DNA upon repair synthesis (34, 35). The displaced single-stranded DNA would be capable of invading and pairing with a nonallelic class I gene, producing heteroduplex DNA (35). The presence of Z-DNA at another position on the invaded, nonallelic gene might serve to limit the extent of recombination by inhibiting branch-migration or the progress of a polymerase through the area of Z-DNA (34). The formation of Z-DNA in class I genes would be favored by the large number of CpG dinucleotides found in the potential Z-DNA tracts, especially if the C residues were methylated (34). Thus, frequent Z-DNA structures in class I genes could account for the relatively short recombination events observed in the generation of the K^b mutants. A role for Z-DNA in modulating recombination between globin genes has been postulated (34). It is relevant that an eukaryotic enzyme has been found in mitotic cells that promotes homologous pairing and the uptake of single-stranded DNA by supercoiled DNA and has strong affinity for Z-DNA (36).

Genealogical analysis of the bm9, bm6, and bm23 mutants provides evidence that the recombination events that generated these mutants occurred during the mitotic amplification of the germ-cell line, rather than during meiosis or in the zygote. That several identical mutant mice of both sexes were produced by the same parents can only be explained by mitotic recombination. Thus, at least some, if not all, K^b mutants have been generated by mitotic recombination. Interestingly, when the studies of D. W. Bailey, I. K. Egorov, H. I. Kohn, and R. W. Melvold on spontaneous and

induced histocompatibility mutants are evaluated collectively, a large percentage of the combined 230 histocompatibility mutants were detected in litters with two or more identical mutant mice (8). It is relevant that mitotic recombination between homologous sequences has been described in lower eukaryotes and in mammalian cells in culture, as has an enzyme capable of mediating such events (36–39).

The K^b mutants may be generated by double, reciprocal crossing-over between K^b and a donor gene or by a nonreciprocal recombination event, such as gene conversion. Our inability to recover all of the products of mammalian recombination events precludes us from distinguishing between these two processes. It is also uncertain whether the recombination events involve homologous chromosomes, sister chromatids, or a single chromatid. It has been observed that the mutant K^b genes, and their products, are not grossly altered (11, 13–15), which indicates the lack of crossing-over during recombination. This may be explained by the fact that *K* genes are in a different $5' \rightarrow 3'$ orientation on chromosome 17 than are *Qa*-region genes (40, 41), and crossing-over would result in large inversions or acentric and dicentric chromosomes.

Due to amplification of the germ line, the products of mitotic recombination in germ cells have a greater probability of being transmitted to the next generation than meiotic recombinant products. Thus, the unusually high frequency of H-2 mutants may be explained by the increased transmission of the products of mitotic recombination events to the next generation, rather than by an intrinsically high recombination or mutation frequency. This, in addition to positive selection for diversity, might account for the extreme polymorphism of H-2 antigens.

Mitotic recombination, as demonstrated in these studies, may be a more widespread phenomenon than previously thought. For example, mitotic recombination between class I genes would generate somatic variants which could sensitize the immune system, leading to the expansion of the alloreactive repertoire. This, in turn, would develop the potential for crossreactions with foreign antigens in the context of self MHC (42). Further, recombination in somatic tumor cells may lead to the expression of novel class I products that can affect the host–tumor relationships and the progress of the neoplasm (43).

The finding that mitotic recombination generates K^b mutants adds another dimension to our understanding of the forces that promote diversification of *H-2* genes and conservation of *Qa* and *Tla* genes. Short recombination events between nonallelic genes, as described for the K^b mutants, would diversify the MHC. On the other hand, longer recombination events would homogenize sequences within the MHC. Crossing-over during recombination is another variable that would affect the level of sequence conservation and diversification. Therefore, the characteristics of *H-2*, *Qa*, and *Tla* genes are the result of the interplay of many parameters, and mitotic recombination in germ cells adds another variable that can influence the concerted evolution and diversification of class I genes.

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