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Genetics

Molecular studies of murine mutant BALB/c-*H-2*^{dm2} define a deletion of several class I genes including the entire *H-2L*^{d} gene

(major histocompatibility complex/*H-2* mutation)

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ABSTRACT **Inbred mouse strains carrying spontaneous mutations in class I genes have been extremely informative in studies of the genetic mechanisms generating polymorphism in the major histocompatibility gene complex. In this report, we determine the molecular basis of the spontaneous loss mutation in BALB/c-*H-2*^{dm2} mice, which were previously shown not to express L^{d} antigens while maintaining normal expression of two other class I antigens, K^{d} and D^{d}. We show BALB/c-*H-2*^{dm2} mice do not transcribe detectable levels of *L*^{d} mRNA, indicating they do not produce a truncated L^{d} molecule as previously reported. Furthermore, in Southern blot comparisons using a series of low-copy genomic probes, the deletion was found to be approximately 140 kilobases and include the entire *L*^{d} gene along with three or more other class I genes mapping between *D*^{d} and *L*^{d}. These data represent direct genetic evidence for a spontaneous contraction in the genes encoding class I histocompatibility antigens, which in this case probably resulted from the misalignment of the 3′ flanking regions of the *D*^{d} and *L*^{d} genes.**

The major histocompatibility complex (MHC) of the mouse, designated *H-2*, is located on chromosome 17 and contains a cluster of genes that encode products of immunological importance. The MHC products in one group, the classical transplantation antigens or class I molecules, elicit allograft rejection and are detectable by alloantibodies (1). These transplantation antigens are encoded by the *K* and *D* regions of the MHC and are found on the cell surface as transmembrane glycoproteins of 45,000 daltons noncovalently associated with β_2-microglobulin. These molecules are present on virtually all nucleated cells and express an extraordinarily high level of genetic polymorphism. This polymorphism is thought to be imperative for their physiological function in immune surveillance against virus-infected cells (2). In addition, recent studies suggest that the genetic mechanisms operative in the generation of class I heterogeneity may also be operative in neoplastic cells, resulting in the expression of altered class I molecules as tumor-specific antigens (3).

Class I genes present in the *D* region can be used to distinguish different mouse strains in terms of both their polymorphism and their total number. For example, in the MHC of BALB/c (*H-2*^{d}) mice, there are at least five *D* region class I genes (4), while in C57BL/10 (*H-2*^{b}) (5) and AKR (*H-2*^{k}) (4) mice only one *D* region class I gene has been found. In contrast, the *K* region of *b* and *d* haplotypes has been shown to contain two class I genes, *K* and *K1* (5, 6). The *K*^{b} and *K*^{d} genes are believed to be responsible for the serological and functional properties that have been ascribed to the *K* region, whereas the *K1* genes may not be expressed (7–9). The remaining class I genes (≈25) map to the *Qa/Tla* region (7). Of the *K* and *D* region class I gene products found in BALB/c, three have been studied by serological and biochemical techniques. These are encoded by the *K*^{d}, *D*^{d}, and *L*^{d} genes, which have been isolated and characterized (9–13). Other serological specificities, notably, M^{d}, R^{d}, and L2^{d}, have been identified, but neither their genetic nor their chemical basis has been ascertained (14–16).

Mouse strains carrying mutations within their MHC have made it possible to gain valuable information on the organization, function, and evolution of the class I genes (17). Of the several mutant strains detected by skin graft rejection, those with mutations in the *K*^{b} gene have been studied most extensively. Data on these mutants suggest that gene conversion is the major progenitor of polymorphism within the *K* region and perhaps the *D* region. Not all of the aforementioned mutants detected in skin graft rejection studies carry mutations resulting from putative gene conversion events. For example, the *D* region product of B10.D2-*H-2*^{dm1} mutant mice (dm1) was recently shown, at the protein (18) and the nucleotide (19) levels, to be a fusion product of the 5′ end of the *D*^{d} gene and the 3′ end of the *L*^{d} gene. However, it is difficult to draw implications of its importance for MHC evolution because it was detected in a mouse treated with the mutagen diethyl sulfate (20). Another interesting mutant that was also not the result of a gene conversion event but was uninduced (i.e., spontaneous) is BALB/c-*H-2*^{dm2} (dm2). The *dm2* mutation is of the "loss type," since dm2 mice reject parental BALB/c skin grafts, whereas dm2 skin grafts are *not* rejected by BALB/c recipients (21). Genetic mapping studies localized the *dm2* mutation in the *D* region, and chemical (22) and serologic (23) studies showed that dm2 mice fail to express a class I molecule expressed by BALB/c mice. Furthermore, the *D* region product not expressed by dm2 mice was found to be distinct from the D^{d} molecule and was therefore given the unique designation L^{d}. Chemical (24) and functional (25) analyses showed L^{d} molecules to be comparable to other *K* and *D* region products.

To ascertain the genetic basis for the failure of dm2 mice to express *L*^{d}, we describe here a series of molecular characterizations of the *D* regions of dm2 and BALB/c mice. Our findings demonstrate that no detectable *L*^{d}-specific RNA transcripts are present in dm2 mice as measured by single-copy oligonucleotide probes. Furthermore, genomic probes derived from the 5′ or 3′ flanking region of *L*^{d} or from *L*^{d} exonic sequences fail to hybridize to sequences corresponding to *L*^{d} in dm2 DNA, indicating the entire gene has been deleted. Data are also presented concerning the size of the deletion and the inclusion of at least four class I genes in this deletion. The evolutionary implications of these results are discussed.

MATERIALS AND METHODS

Mice. BALB/c, dm2, B10.D2, and dm1 mice were obtained from Donald C. Shreffler's animal facility at Washington University School of Medicine (St. Louis, MO).

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Abbreviations: dm1, B10.D2-*H-2*^{dm1}; dm2, BALB/c-*H-2*^{dm2}; kb, kilobase(s); MHC, major histocompatibility complex; RFLP, restriction fragment length polymorphism.

Hybridization Probes. Probe 1: 0.4-kilobase (kb) *Xba* I–*Bam*HI fragment covering the 5′ flanking and part of exon 1 of the L^d gene. Probe 2: 1.2-kb *Bam*HI–*Pst* I fragment covering exons 1, 2, and most of exon 3 of L^d. Probe 3: 0.3-kb *Pst* I–*Bgl* II fragment covering intron 4, exon 5, and intron 5 of L^d. Probe 3A: 0.2-kb *Sau*3AI–*Sau*3AI fragment from probe 3. Probe 4: 0.9-kb *Sst* I–*Sst* I fragment 2.1 kb 3′ of L^d. Probe 5: 1.5-kb *Bam*HI–*Bam*HI fragment from cosmid 12.1 (19). Probe 6: 1.4-kb *Bam*HI–*Bam*HI fragment from cosmid 12.1 (19). Probe 7: 1.7-kb *Bam*HI–*Bam*HI fragment from cosmid 12.1 (19). Probes were isolated in low-melting-point agarose (Bethesda Research Laboratories) and labeled by the random hexamer technique (26). Oligonucleotide probes L^d-C2A (5′-TTCACCTTTAGAGGGGTGATG-3′) and D^d-C2A (5′-ATCACCTTCAGGTCTGCGGTGATG-3′) are complementary to the 5′ variable regions of the L^d and D^d *C2* exons, respectively.

RNA Blots. Cellular RNA was prepared from liver and spleen by a modification of the hot phenol extraction procedure of Soeiro and Darnell (27). RNA (50 μg per lane) was size fractionated by electrophoresis in 1% agarose gels containing 20 mM boric acid (pH 8.3), 0.5 mM EDTA, and 3% (wt/vol) formaldehyde, then transferred to GeneScreen (New England Nuclear) for hybridization analysis. Prehybridization was performed for 4 hr in 7% sodium dodecyl sulfate ($NaDodSO_4$), 20 mM sodium phosphate (pH 7.0), 10× Denhardt's solution (0.2% bovine serum albumin/0.2% Ficoll/0.2% polyvinylpyrrolidone), 5× SSC (0.75 M NaCl/0.075 M sodium citrate), and heat-denatured salmon sperm DNA at 200 μg/ml at either 57°C (for the L^d-C2A probe) or 65°C (for the D^d-C2A probe). Hybridization was performed overnight at the respective prehybridization temperatures after addition of the 5′-^{32}P-labeled oligonucleotide probe and dextran sulfate (10%, wt/vol). After hybridization, the filters were washed for 1 hr at the respective prehybridization temperatures in 3× SSC/5% $NaDodSO_4$/20 mM sodium phosphate (pH 7.0)/10× Denhardt's solution. Subsequently, the filters were washed at the same temperature for an additional 1 hr in 1× SSC/1% $NaDodSO_4$/20 mM sodium phosphate (pH 7.0)/10× Denhardt's solution. The filters were then exposed to Kodak X-Omat AR film between two intensifying screens.

Southern Blots. High molecular weight DNA was prepared according to a modified procedure of Chang *et al.* (28). Ten to fifteen micrograms of DNA was fractionated by electrophoresis in 0.7% agarose gels containing ethidium bromide at 0.5 μg/ml then transferred to nitrocellulose (Schleicher & Schuell). Prehybridization was performed for 2 hr in 6× SSC/5× Denhardt's solution/20 mM sodium phosphate buffer (pH 7.0)/4 mM EDTA/200 μg of heat-denatured salmon sperm DNA per ml/0.2% $NaDodSO_4$ at 68°C. Hybridizations were performed overnight at 68°C with labeled probes. After hybridization, the nitrocellulose was washed for 1 hr at 68°C in 0.5× SSC. The blots were then exposed to X-Omat AR film between two intensifying screens. Subsequently, 0.1× SSC was used to wash the blot for an additional 1 hr at 68°C, after which the blot was placed on film between two intensifying screens.

RESULTS

Failure to Detect L^d Gene Transcripts. To detect the presence of L^d-specific and D^d-specific RNA transcripts in dm2 mice, two 24-residue (24-mer) oligonucleotide probes corresponding to the 5′ variable region of the *C2* exon from the L^d gene or the D^d gene (L^d-C2A and D^d-C2A, respectively) were used. The specificities of the probes were confirmed by using plasmids and cosmids containing the L^d or D^d gene (data not shown). In Fig. 1*A*, both spleen and liver RNA derived from BALB/c possess a transcript that hybridizes with the L^d-C2A oligonucleotide probe of L^d. In contrast, this transcript is not present in either spleen or liver RNA preparations from dm2 mice. Shown in Fig. 1*B* is a blot prepared in an identical manner as the blot shown in Fig. 1*A*, with the exception that it was hybridized with the D^d-C2A oligonucleotide probe. This hybridization served as a control for the RNA preparations from dm2 and confirmed the presence of a D^d transcript in both BALB/c and dm2 mice. Since a probe to a conserved domain such as *C2* was used, this makes it unlikely that an L^d transcript, either normal or truncated, would be missed in these blotting procedures. Thus, dm2 mice do not appear to produce a truncated L^d-gene product as previously reported (29). To determine if this absence of an L^d transcript was due to a deletion of the L^d gene, DNA isolated from dm2 mice was subjected to Southern blot analyses.

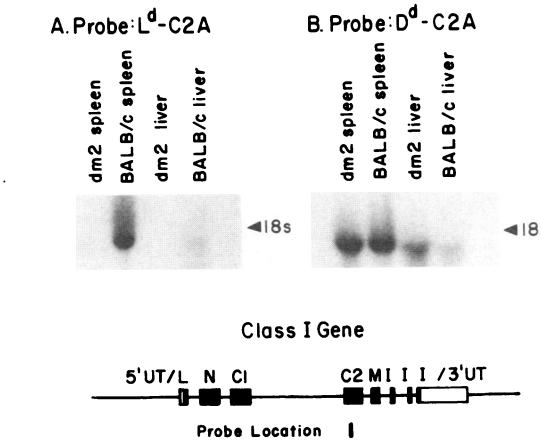

FIG. 1. Blot hybridization analysis of cellular RNA extracted from the spleen and liver of BALB/c and dm2 mice. The blots were hybridized with the L^d-specific (L^d-C2A, *A*) or D^d-specific (D^d-C2A, *B*) oligonucleotide probe. The bands shown were the only ones apparent in these blots. The bottom diagram depicts a typical class I gene with its eight exons denoted with solid boxes. The location of the oligonucleotide probes is indicated by the bar beneath the C2 exon. UT, untranslated region.

The L^d Gene Is Deleted in dm2. For Southern blot analyses, probes derived from exons, the 5′ flanking/untranslated region, and the 3′ flanking region of the L^d gene were used to determine if this gene is present in dm2 mice (see Fig. 2). In Fig. 3*A*, Southern blots of *Bgl* II-digested genomic liver DNA hybridized with exonic probe 2 are shown. A 6.6-kb fragment present in BALB/c DNA that contained approximately 3.9 kb of the 5′ flanking region of the L^d gene in addition to 2.7 kb of DNA comprising the entire first three exons and part of

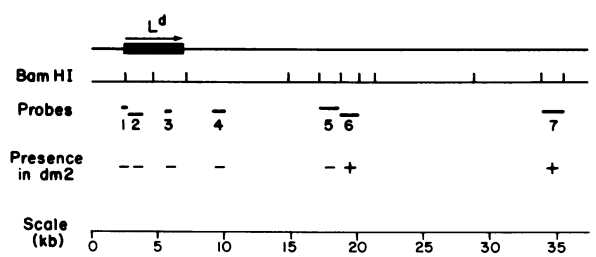

FIG. 2. Location of probes isolated from the BALB/c L^d gene and its flanking regions. The top line shows the L^d gene as a solid box with the arrow above indicating its 5′-to-3′ orientation of transcription. The locations from which the probes were derived are indicated by solid bars beneath the *Bam*HI map. The presence or absence of a particular DNA sequence in the dm2 genome is indicated by a plus or minus, respectively. The *Bam*HI restriction sites are taken from ref. 19.

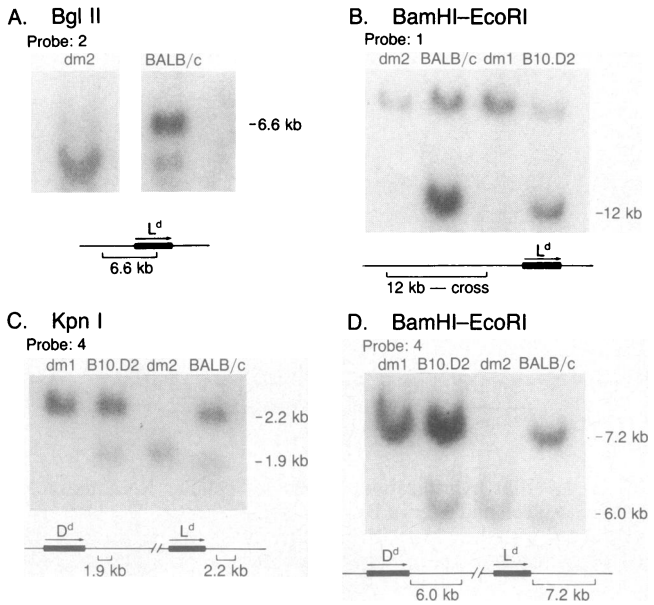

FIG. 3. Southern blot analysis to demonstrate the absence of the L^d gene. (*A*) *Bgl* II-digested DNA hybridized to probe 2. (*B*) *Bam*HI–*Eco*RI-digested DNA with probe 1. (*C* and *D*) Probe 4 hybridized with *Kpn* I- or *Bam*HI–*Eco*RI-digested DNA, respectively. In *C*, the 1.9-kb band in this blot appears slightly smaller in BALB/c than B10.D2 or dm1. This difference, however, was not observed in additional blots run under these same conditions. The L^d- and D^d-specific fragments were identified by their coincidental migration with fragments obtained from appropriate cosmids provided by L. Hood. In *A* and *B*, 2 of approximately 10 bands are shown, whereas in *C* and *D*, all bands are shown. The locations of the L^d- and D^d- specific fragments are shown in the diagrams below the blots.

the fourth exon of the L^d gene is absent from dm2 DNA. This result demonstrated that the 5′ half of the L^d gene is deleted in dm2 mice. The 5′ flanking/untranslated probe, probe 1, did not detect an 11-kb *Kpn* I band corresponding to L^d in DNA from either dm2 or dm1 mice, while the band was detected in B10.D2 and BALB/c DNA (data not shown). The 17-kb D^d band was present in each of the haplotypes tested, as expected (data not shown). The dm1 mutant served as a control, because it has recently been shown that the 5′ end of the L^d gene was deleted from its genome (19). Interestingly, probe 1 also detected a cross-hybridizing 12-kb *Bam*HI–*Eco*RI fragment that mapped approximately 17 kb 5′ of the L^d gene as shown in Fig. 3*B*. This 12-kb L^d-specific band was present in DNA from BALB/c and B10.D2 mice but not in DNA from dm1 or dm2 mice. The location of the cross-hybridizing fragment was confirmed by using the same restriction enzymes to digest cosmid 59.2 (19), which includes the 5′ flanking region of the L^d gene. The reason for the cross-hybridization in this region is not known. However, it is tempting to speculate that there may be some regulatory sequences in this region, because the probe used to detect this fragment is derived from an area that contains the 5′ promoter sequences of the L^d gene. Under the conditions used in Fig. 3*B*, the L^d band corresponding to the region from which the probe was derived could not be differentiated from the D^d band due to a lack of restriction fragment length polymorphisms (RFLPs) in this region.

As shown in Fig. 3 *C* and *D*, a 3′ flanking probe, probe 4, detected RFLPs between the L^d gene and the D^d gene in blots containing either *Kpn* I- or *Bam*HI–*Eco*RI-digested DNA. In these hybridizations, the DNA from BALB/c and B10.D2 mice was found to possess both L^d- and D^d-specific bands, whereas DNA from dm1 mice possessed only the L^d-specific band and DNA from dm2 mice, only the D^d band. Probe 4 established that the *dm2* deletion extends through the L^d gene and into its 3′ flanking region. These results establish that the entire L^d gene has been deleted in dm2 mice.

Number of Genes Deleted. To estimate the number of class I genes deleted in the *dm2* mutation, two probes derived from the transmembrane exon and its flanking introns (probes 3 and 3A) were used. This approach assumes that each band found in BALB/c that is missing in dm2 represents a different *D* region gene (Fig. 4). Close attention was given to the dm2 lane to confirm that no new bands were found that were absent in the BALB/c lane. These new bands would have suggested an alteration of restriction sites without the loss of a gene. On Southern blots using liver DNA digested with *Sst* I, these membrane probes detected as many as four fragments in BALB/c that were absent in dm2. These four fragments were approximately 19, 2.9, 1.8, and 0.5 kb in length and were also found to be missing in DNA from dm1 (data not shown). Since the L^d and D^d genes showed no RFLPs under these conditions (cosmid controls are not shown in Fig. 4), the loss of L^d-specific fragments was not detected in this comparison. Stephan *et al.* (4) have recently shown in BALB/c ($H\text{-}2^d$) mice the presence of at least five *D* region genes, ordered and designated, D^d, $D2^d$, $D3^d$, $D4^d$, and L^d. Our results show four fragments that differ between BALB/c and dm2, excluding the D^d and L^d genes. Therefore, there may be an additional *D* region gene present in BALB/c that has yet to be defined. This additional D region gene may have been missed previously due to the complex task of analyzing restriction maps of *D* region cosmids (see ref. 30). Because these four fragments gave weak hybridization to our membrane probe, they may have been overlooked if the proper probe was not used under favorable conditions. Alternatively, our approach could have overestimated the number of genes if *Sst* I cut within the hybridization region. It should be noted that it still has to be determined whether these genes located between D^d and L^d are functional.

Length of the dm2 Deletion. To determine the boundaries of the *dm2* deletion, several probes were derived from the 3′ flanking region of L^d and used to map the recombination site in DNA isolated from dm2 mice. As shown in Fig. 5, the telomeric deletion site can be narrowed down to a small

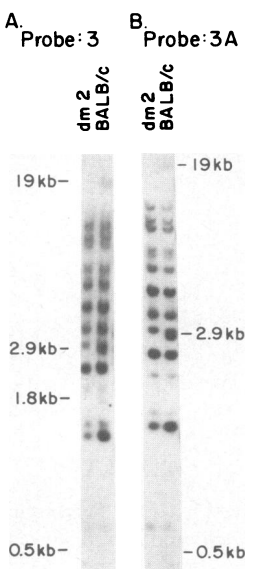

FIG. 4. Southern blot analysis of *Sst* I-digested DNA with probes 3 and 3A. The four bands indicated in *A* are potential *D* region genes that differ between BALB/c and dm2. Although the 0.5-kb band is faint, it was reproducibly seen in parallel experiments. The results in *B* are shown to make it apparent that the band at 2.9 kb is actually absent from dm2 DNA.

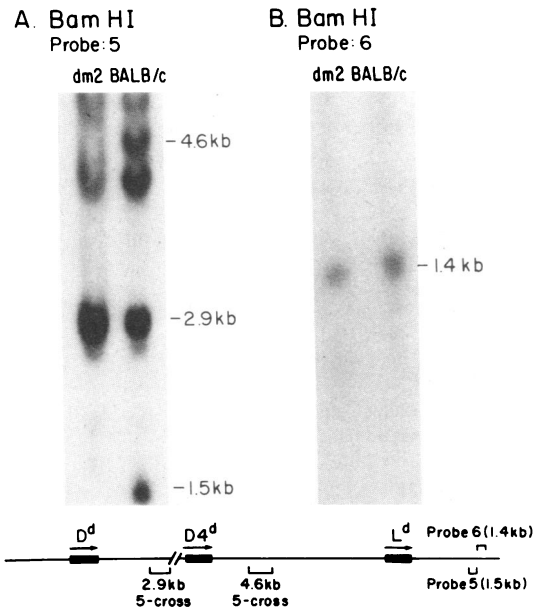

FIG. 5. Southern blot analysis to determine the size and location of the deletion in dm2. BALB/c and dm2 DNA digested with *Bam*HI were hybridized with probes 5 and 6 in *A* and *B*, respectively. Approximately four other hybridization bands present in each lane of *A* are not shown. The bands shown in *B* were the only ones observed. The diagram at the bottom shows the locations of probes 5 and 6 as well as the location of the two other fragments that cross-hybridize with probe 5.

stretch of DNA (see Fig. 2). In Fig. 5*A*, probe 5 showed strong hybridization to its corresponding 1.5-kb BALB/c *Bam*HI fragment but did not hybridize to a similar dm2 fragment. No new bands appeared in the lane containing DNA from dm2 mice. This result implied that the *dm2* deletion extends into this 3′ flanking region. In Fig. 5*B*, probe 6, derived immediately 3′ of probe 5, gave bands with DNA from both BALB/c and dm2, suggesting that these haplotypes are identical at this location. Probe 6, therefore, detected the telomeric end of the *dm2* deletion. This implied that the recombination site must be centromeric of this fragment, in the 1.5-kb *Bam*HI hybridizing fragment. Another 3′ flanking probe located ≈27 kb 3′ of L^d (probe 7) hybridized to its corresponding 1.7-kb *Bam*HI fragment in DNA isolated from both BALB/c and dm2 mice (data not shown). This confirmed that the genomes of BALB/c and dm2 are identical at this distance 3′ of L^d. Therefore, these results established that the *dm2* mutation extends approximately 11 kb 3′ of the L^d gene.

Two interesting observations were noted with probe 5. First, this probe cross-hybridized with a 4.6-kb fragment located 7 kb 3′ of $D4^d$, in both BALB/c (Fig. 5*A*) and B10.D2 DNA (data not shown). The absence of this fragment in dm1 (data not shown) and dm2 (Fig. 5*A*) DNA is consistent with results describing the extent of the deletions in these mice. Second, the ≈2.9-kb *Bam*HI fragment that cross-hybridized with probe 5 was present in BALB/c, dm2, and B10.D2 DNA but absent from dm1 DNA (Fig. 5*A* and data not shown). This result helped define the centromeric site of recombination in dm2. The location of this fragment is inferred to be between 7.6 and ≈27 kb 3′ of D^d as determined from restriction mapping data from other investigators (4, 30). Our data together with that of Stephan *et al.* (4) predict that the centromeric recombination site is located in a segment of DNA 10.5 to 27 kb 3′ of D^d. Therefore, the dm2 deletion is approximately 140 kb in length.

DISCUSSION

The combined application of nucleic acid techniques and immunogenetic analyses provides new and more sophisticated approaches to address questions regarding the enumeration of class I genes. In this report, we define the molecular boundaries of the *dm2* mutation and establish the genetic basis for the failure of dm2 mice to express L^d antigens. Previous studies by Stephan *et al.* (4) have shown that dm2 mice are missing the genetic material between the D^d and L^d genes. We confirmed their observation that additional class I genes telomeric of the D^d gene are missing in dm2 mice. Furthermore, we showed that this deletion extends through the entire L^d gene, totaling approximately 140 kb. In RNA blot hybridization analyses using single-copy oligonucleotide probes, liver and spleen cells of dm2 mice did *not* contain detectable L^d transcripts, whereas D^d transcripts were readily observed. Therefore, these combined data using Southern and RNA hybridizations unequivocally demonstrate that dm2 mice have a deletion of the entire L^d gene, in contrast to the results of Robinson (29).

The location of the *dm2* mutation suggests that it occurred as the result of a meiotic misalignment of the D^d gene with the L^d gene, followed by a recombination event in the 3′ flanking regions of these mismatched genes (see Fig. 6). This putative unequal crossover in dm2 would have deleted the entire L^d gene and all genetic material between the D^d and L^d genes, thus leaving only the D^d gene in this region. Our hybridization data along with the hybridization and restriction endonuclease data of Stephan *et al.* (4) suggest that the 5′ and 3′ flanking regions of the L^d and D^d genes are highly homologous. These regions of high homology would allow misalignment of sister chromatids, creating the potential for unequal crossover events deleting the genetic material between the D^d and L^d genes. The *dm1* and *dm2* mutations are probable examples of such events.

These molecular characterizations of the dm2 mutant provide direct genetic evidence for a spontaneous contraction in the number of *D* region genes. Whereas parental BALB/c mice contain at least five *D* region class I genes, dm2 mice have only one, the D^d gene. The molecular characterizations of the *D* region genes of the *b* (5) and *k* (4) haplotypes also suggest the presence of only a single class I gene. In apparent contrast to these haplotypes, the *q* (31, 32) and *w16* (33) haplotypes express multiple, chemically distinct, *D* region-encoded antigens that are presumed to be products of separate genes. Molecular analysis of the D^q and D^{w16} region genes should provide important information for tracing the evolution of class I genes.

Similar to the *D* region, a spontaneous deletion of class I genes in the *Qa* region has been noted (34); furthermore, the number of *Qa* region class I genes differs in the $H\text{-}2^d$ and $H\text{-}2^b$

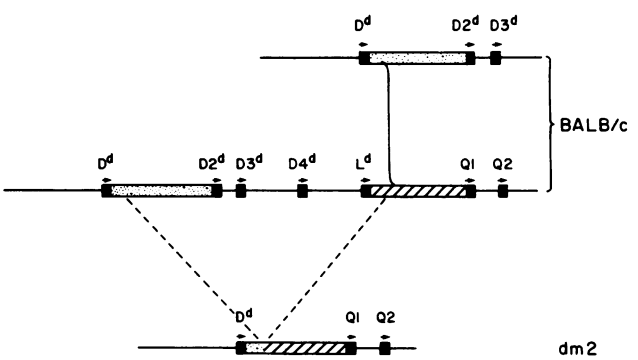

FIG. 6. Proposed unequal crossover event that led to the *dm2* mutation. The stippled and hatched areas represent the highly homologous 3′ flanking regions of the D^d and L^d genes, respectively.

haplotypes (5, 7, 30). The haplotype differences in the number of *D* and *Qa* region genes are in striking contrast to what has been found thus far in the *K* region. The K^b and K^d regions have each been found to contain two class I genes, *K* and a putative pseudogene referred to as *K1* (5–7, 9). These findings perhaps indicate that there are different genetic constraints imposed on the evolution of class I genes from different regions. Though the high level of polymorphism observed in *K* and *D* region class I genes appears to be driven by gene conversion-like events using a variety of class I genes as donors (35), the frequency of or mechanisms involved in those events relative to *K* or *D* region genes could differ. Meanwhile, the *D* and *Qa* regions are undergoing expansion and contraction of their class I genes, thus providing new or different candidates for participation in these conversion-like events. Although many details have yet to be filled in, each class I region appears to play a unique and critical role in the generation and maintenance of the polymorphism associated with the genes encoding histocompatibility antigens.

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1. Klein, J. (1979) *Science* **203,** 516–521.
2. Zinkernagel, R. M. & Doherty, P. C. (1974) *Nature (London)* **248,** 701–702.
3. Philipps, C., McMillan, M., Flood, P. M., Murphy, D. B., Forman, J., Lancki, D., Womack, J. E., Goodenow, R. S. & Schreiber, H. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5140–5144.
4. Stephan, D., Sun, H., Fischer-Lindahl, K., Meyer, E., Hammerling, G., Hood, L. & Steinmetz, M. (1986) *J. Exp. Med.* **163,** 1227–1244.
5. Weiss, E. H., Golden, L., Fahrner, K., Mellor, A. L., Devlin, J. J., Bullman, H., Tiddens, H., Bud, H. & Flavell, R. A. (1984) *Nature (London)* **310,** 650–655.
6. Steinmetz, M., Stephan, D. & Lindahl, K. F. (1986) *Cell* **44,** 895–904.
7. Winoto, A., Steinmetz, M. & Hood, L. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3425–3429.
8. Goodenow, R. S., McMillan, M., Orn, A., Nicolson, M., Davidson, N., Frelinger, J. A. & Hood, L. (1982) *Science* **215,** 677–679.
9. Mellor, A. L., Golden, L., Weiss, E., Bullman, H., Huerst, J., Simpson, E., James, R. F. L., Towsend, A. R. M., Taylor, P. M., Schmidt, W., Ferluga, J., Leber, L., Santamaria, M., Atfield, G., Festenstein, H. & Flavell, R. A. (1982) *Nature (London)* **298,** 529–534.
10. Evans, G. A., Margulies, D. H., Camerini-Otero, D., Ozato, K. & Seidman, J. G. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 1994–1998.
11. Goodenow, R. S., McMillan, M., Nicolson, M., Sher, B. T., Eakle, K., Davidson, N. & Hood, L. (1982) *Nature (London)* **300,** 231–237.
12. Moore, K. W., Sher, B. T., Sun, Y. H., Eakle, K. A. & Hood, L. (1982) *Science* **215,** 679–682.
13. Margulies, P. H., Evans, G. A., Ozato, K., Camerini-Otero, R. D., Tanaka, K., Appella, E. & Seidman, J. G. (1983) *J. Immunol.* **130,** 463–470.
14. Demant, P. & Ivanyi, D. (1981) *Nature (London)* **290,** 146–149.
15. Hansen, T. H., Ozato, K., Melino, M. R., Coligan, E. J., Kindt, T. J., Jandinski, J. & Sachs, D. H. (1981) *J. Immunol.* **126,** 1713–1716.
16. Ivanyi, D. & Demant, P. (1982) *Immunogenetics* **15,** 467–476.
17. Nairn, R., Yamaga, K. & Nathenson, S. G. (1980) *Annu. Rev. Genet.* **14,** 241–277.
18. Burnside, S. S., Hunt, P., Ozato, K. & Sears, D. W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5204–5208.
19. Sun, H., Goodenow, R. S. & Hood, L. (1985) *J. Exp. Med.* **162,** 1588–1602.
20. Egorov, I. K. (1967) *Genetika* **3,** 136–143.
21. McKenzie, I. F. C., Morgan, G. M., Melvold, R. W. & Kohn, H. I. (1977) *Immunogenetics* **4,** 333–347.
22. Hansen, T. H., Cullen, S. E., Melvold, R., Kohn, H., Flaherty, L. & Sachs, D. H. (1977) *J. Exp. Med.* **145,** 1550–1558.
23. Hansen, T. H. & Sachs, D. H. (1978) *J. Immunol.* **121,** 1469–1472.
24. Coligan, J. E., Kindt, T. J. H., Nairn, R., Nathenson, S. G., Sachs, D. H. & Hansen, T. H. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1134–1138.
25. Levy, R. B. & Hansen, T. H. (1980) *Immunogenetics* **10,** 7–17.
26. Feinberg, A. P. & Vogelstein, B. (1980) *Anal. Biochem.* **132,** 6–13.
27. Soeiro, R. & Darnell, J. E. (1969) *J. Mol. Biol.* **44,** 551–562.
28. Chang, H., Omitrovsky, E., Hieter, P. A., Mitchell, K., Leder, P., Turoczi, L., Kirsch, I. R. & Hollis, G. F. (1986) *J. Exp. Med.* **163,** 425–435.
29. Robinson, P. J. (1982) *Immunogenetics* **15,** 330–340.
30. Steinmetz, M., Winoto, A., Minard, K. & Hood, L. (1982) *Cell* **28,** 489–498.
31. Lillehoj, E. P., Hansen, T. H., Sachs, D. H. & Coligan, J. E. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2499–2503.
32. Chatterjee-Das, S., Berg, S. K. & Sachs, D. H. (1985) *Transplant. Proc.* **17,** 722–724.
33. Lillehoj, E. P., Walsh, W. D., Potter, T., Lee, D. R., Coligan, J. E. & Hansen, T. H. (1984) *J. Immunol.* **133,** 3138–3142.
34. Flaherty, L., DiBiase, K., Lynes, M. A., Seidman, J. G., Weinberger, O. & Rinchik, E. M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1503–1507.
35. Nathenson, S. G., Geliebter, J., Pfaffenbach, G. M. & Zeff, R. A. (1986) *Annu. Rev. Immunol.* **4,** 471–502.