Molecular Genetic Analysis of the mld' Mouse: A Spontaneous Revertant at the mld Locus Containing a Recombinant Myelin Basic Protein Gene

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Manuscript received September 9, 1991
Accepted for publication October 24, 1991

ABSTRACT
The mld mutation is a complex genetic lesion affecting the myelin basic protein (MBP) locus in the mouse. The mutation consists of a variety of DNA rearrangements including; tandem duplication of the MBP structural gene, partial inversion of the 3' end of the upstream gene copy, duplication of a region flanking the rearrangement junction in the upstream copy and insertion between the two gene copies of a segment of extraneous DNA not associated with the wild-type MBP locus. The net result of the mutation is a dysfunctional MBP locus. Homozygous mld/mld mice produce very little MBP and consequently very little myelin. They exhibit a clinical phenotype characteristic of hypomyelination (shaking, convulsions). We have discovered a revertant mld mouse which does not exhibit clinical symptoms of hypomyelination. Genetic analysis indicates that the reversion is allelic to mld. We have designated the revertant locus mld'. Restriction analysis of mld' genomic DNA indicates that there is a single intact MBP gene. Analysis of various junction regions using the polymerase chain reaction indicates that the single MBP gene in mld' is derived by recombination from the 5' end of the upstream gene and the 3' end of the downstream gene. Studies on MBP expression in mld' mice indicate that the developmental regulation, level of expression and pattern of post-transcriptional processing of MBP gene products in mld' are similar to wild type. These results indicate that the recombinant MBP gene in mld' is fully functional. From this we infer that the MBP-deficient phenotype of the original mld mutant is attributable to the complex rearrangements in the upstream gene copy which render the locus dysfunctional. Elimination of the rearranged DNA in mld' restores function to the locus and ameliorates the mutant phenotype.

Molecular genetic analysis of mutations that disrupt gene function can provide insights into mechanisms of gene expression. However, if the genetic lesion is complex it may be unclear which aspect of the mutation is responsible for the mutant phenotype. In such cases it is often informative to analyze revertants in which the mutant phenotype has been cured by a second mutation. If the reversion affects a particular aspect of the original genetic lesion this implicates that aspect of the original genetic lesion as a cause of the mutant phenotype.

The myelin deficient (mld) mutation in the mouse (DOOLITTLE and SCHWEIKART 1977) is an example of a complex genetic lesion that disrupts gene expression. As diagrammed in Figure 1, the mutation consists of four distinct DNA rearrangements at the myelin basic protein (MBP) locus on chromosome 18. First, the MBP gene is tandemly duplicated (AKOWITZ et al. 1987; POPKO et al. 1987). Second, the upstream gene copy is partially rearranged such that the 3' end of the gene is inverted relative to the 5' end (POPKO, PUCKETT and HOOD 1988). Third, a region of DNA flanking the rearrangement junction is duplicated (OKANO et al. 1991). Fourth, a segment of non-MBP locus DNA is inserted between the upstream and downstream genes (OKANO et al. 1988, 1991; OKANO, IKENAKA and MIKOSHIBA 1988). As a result of this combination of DNA rearrangements the MBP locus in mld spans approximately 100 kb compared to approximately 33 kb in wild type.

The complex genetic lesion in mld results in a dysfunctional MBP locus. Homozygous mld/mld mice produce very little MBP and consequently very little myelin. They exhibit a clinical phenotype characteristic of CNS hypomyelination (shaking, convulsions) which becomes apparent approximately 2 weeks after birth. Heterozygous +/-mld mice display no obvious clinical phenotype but do express reduced levels of MBP compared to wild type indicating that the mutant allele is codominant with the wild-type allele at the biochemical level (AKOWITZ et al. 1987; ROCH et al. 1986). This suggests that the mld mutation is cis-acting, inhibiting the function of the MBP locus on the mutant chromosome without affecting the function of a wild-type MBP gene on a separate chromosome.

The structure of the MBP locus in wild type and mld mouse DNA. The diagram summarizes current understanding of the structure of the MBP locus in mld and wild-type mouse DNA. It is based on published information from several different laboratories. The MBP structural gene is shown as a horizontal rectangle with the positions of the 7 exons indicated by vertical black bars. The triangle represents a segment flanking the inversion which is duplicated in the upstream gene in mld. The thin horizontal line represents chromosome 18 DNA flanking the MBP structural gene sequences. The cross-hatched square represents a segment of extraneous non-MBP DNA that is inserted between the upstream and downstream genes in mld. The positions of various junction sequences referred to in the text are indicated by vertical lines with letters designating the appropriate oligonucleotide primer used to analyze each junction sequence. The positions of BglII restriction fragments that hybridize to a specific BamHI probe (shown as a horizontal black bar) are indicated by horizontal brackets.

1988, 1991; ROCH et al. 1989; TOSIC et al. 1990). However, it cannot express myelin basic protein because transcription through the inversion at the 3' end of the gene will generate antisense RNA disrupting the protein coding region. The apparently intact downstream gene copy is also transcribed (TOSIC et al. 1989, 1990; FREMEAU and POPKO 1990; OKANO et al. 1991). This may represent run-on of transcripts initiated at the upstream gene or independent initiation at the downstream gene. In any case the transcripts that are generated appear to be unstable and do not accumulate in the cytoplasm which may account for the lack of MBP expression in these mice.

There are several possible explanations for the dysfunctionality of the MBP locus in mld. The downstream gene may be intrinsically inactive due to a mutational alteration that has not been detected by the extensive restriction mapping and sequence analysis that have been performed on the locus. Upstream insertion of the rearranged gene copy may disrupt essential regulatory elements of the intact gene copy. Proximity of the rearranged gene copy may inactivate transcription initiation at the downstream intact gene copy by transcriptional interference or by other positional effects. Transcription of the rearranged gene copy may generate antisense RNA products that interfere with expression of the intact gene. Any one of these possibilities could explain the reduced expression of MBP which is observed in mld mice.

While MBP expression is greatly reduced in mld/mld mice, a small amount of MBP (approximately 5% of wild type) does accumulate. One possible explanation for the residual MBP expression is that all mld oligodendrocytes are capable of expressing MBP at a low level. A second possibility is that in a small proportion of the oligodendrocytes the mutant phenotype is somehow suppressed restoring functionality to the MBP locus. Immunohistochemical staining of tissue sections (SHEN et al. 1985) and primary cultures (AKOWITZ et al. 1987) from mld/mld mice indicate that a small proportion of the oligodendrocytes are MBP-positive. This provides evidence in support of the second explanation. However the mechanism of suppression of the mutant phenotype in the MBP-positive oligodendrocytes is unknown.

In the work described in this paper we analyze a revertant mouse, mld', which arose in our colony of mld mice. We have performed genetic studies to determine if the site of the reversion in mld' is linked to the MBP locus, restriction analyses and polymerase chain reaction studies to determine which aspects of the original genetic lesion are affected by the reversion, and biochemical studies to assess the functionality of the MBP locus in mld' animals.

MATERIALS AND METHODS

Animals: The mld mutation originally appeared as an independently occurring mutation at the shri locus on the now extinct MDB/Dt inbred genetic background. mld and shri mutant mice were obtained originally from M. WOLF (University of Massachusetts, Worcester) on a hybrid C57BL/6J-C3H/HeJ genetic background. We have propagated these strains in our laboratory by brother sister mating for several years. The putative mld'/mld proband appeared as a nonshaking male in a litter of 8 animals from mld/mld parents. It is unlikely that it could have arisen through contamination of the breeding stock for several reasons. It was the same size and had the same coat color as the shaking littermates. The mld/mld mice were housed separately from other mutant and wild-type mice. Genetic tests (see RESULTS) indicate that the proband was heterozygous for the mld mutation while its littermates were homozygous mld/mld. No heterozygous mld/+ animals were maintained in the colony at the time the proband arose. Mice were maintained in the Center for Laboratory Animal Care at the University of Connecticut Health Center. Animals were sacrificed by cervical dislocation after ether anesthesia. The cerebral hemispheres, livers and spleens were removed and either processed immediately or frozen in liquid nitrogen and stored at −80°C.

Immunoblotting of MBPs: Homogenates of cerebral hemispheres from mice of 5, 15 and 25 days of age were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by MAEZEL (1971). The separated polypeptides were transferred electrophoretically to nitrocellulose membranes (TOWBIN, STAHELIN and GORDON 1979), incubated with antibody to MBP, prepared as described previously (BARBARESE, BRAUN and CARSON 1977), and phosphatase conjugated secondary antibody, and visualized by incubation with phosphatase substrate.
**Restriiction analysis of MBP gene sequences:** Genomic DNA was isolated from adult mouse liver by the method of BLIN and STAFFORD (1976). Isolated DNA was digested with restriction enzyme, electrophoresed on agarose gels and blotted to charge-modified nylon membrane (Genescreen Plus, Du Pont) as described by REED and MANN (1985). The blots were hybridized with appropriate 32P-labeled hybridization probe and analyzed using the Betascope (Betagen). The data file from the Betascope was transferred to a VAX computer and individual bands were quantitated by a specialized integration program written in VAX fortran. The hybridization probe was a 4.5-kb BamHI fragment representing the region in the MBP gene from approximately 14.5 kb to 19 kb. The probe was prepared as a subfragment from cos138 which is a cosmid containing the entire mouse MBP gene (TAKAHASHI et al. 1985).

**Polymerase chain reaction analysis of recombination junctions:** Oligonucleotide primers flanking the AA’, CD, B’X and X’A’ junctions within the MBP locus in mld DNA (see Figure 1) were synthesized based on published sequence data (OKANO et al. 1988, 1991). The oligonucleotide sequences were as follows:

A 5’CCCTATTTCACATTTGCCGTCATGA3’
A’ 5’AGCCGGGGGCTCACATGGGCTCCTC3’
C 5’GGTTTCCTGGGAAACAGTGAGCTTTGG3’
D 5’TAGAGCCAGCTTTCTGTTACAGTGA3’
B’ 5’AGCTTTGTATTAATAAGCAAGATGTC3’
X 5’GGCTGTTTACACAGATCTAAGACG3’
X’ 5’TTCTGCAACTGTTACGTCCTTTA3’

Appropriate primer pairs were incubated with genomic DNA and Vent DNA polymerase (New England Biolabs) in a programmable temperature controller (MJ Research). Each cycle consisted of melting for 2 min at 94°, annealing for 2 min at 45°, and chain elongation for 2 min at 72°. After 24 cycles aliquots of the reaction were analyzed directly by electrophoresis on 3% NuSieve/1% agarose gels and ethidium bromide staining.

**RESULTS**

**Identification of the mld’ revertant mouse:** Homozygous mld/mld mice display a characteristic clinical hypomyelination phenotype (shaking, uncoordinated movements and convulsions) which first becomes apparent by 12–14 days of age. In a litter of 8 mice produced from homozygous mld/mld parents, we discovered a single male offspring that did not display the characteristic shaking mld phenotype. We hypothesized that the nonshaking animal was a spontaneous revertant which still carried the mld mutation but in which a second mutation had occurred restoring function to the MBP locus on one or both chromosomes allowing myelination to take place. To test this hypothesis the putative revertant was backcrossed to mld/mld females (Figure 2). Approximately half the progeny displayed the shaking mld/mld phenotype and half did not shake. This indicates that the revertant animal carried the mld mutation on at least one chromosome and that whatever caused the revertant phenotype was transmitted to half the offspring.

The nonshaking offspring of the first backcross were intercrossed by brother sister mating. Most of the progeny of these crosses did not shake but a small proportion did shake. These results are consistent with the hypothesis that the revertant mouse carried the original mld mutation plus a second mutation that reversed the effects of mld and that segregated as a single Mendelian trait. The putative genotypes of these offspring are indicated in Figure 2.

The nonshaking intercross progeny were backcrossed individually to mld/mld animals to test for their ability to produce shaking mld/mld offspring. Animals which produced no shaking offspring among at least 20 progeny in at least three separate litters were assumed to be homozygous for the reversion mutation and were designated mld'/mld'. These putative homozygous mld'/mld' animals were inter-

![Figure 2](image-url). Partial pedigree illustrating the isolation of the mld' mutant mouse. A nonshaking (NS) male mouse, designated the proband, was discovered in a litter of 8 offspring of homozygous mld/mld parents. The proband (presumed genotype mld/mld) was backcrossed to several homozygous female mld/mld mice and generated approximately equal numbers of shaking (S) (presumed genotype mld/mld) and nonshaking (NS) (presumed genotype mld'/mld) progeny. The nonshaking progeny were intercrossed by brother-sister mating. The offspring were predominantly nonshaking (presumed genotypes mld'/mld', mld'/mld) with a small proportion of shaking animals (presumed genotype mld/mld). The nonshaking F1 animals were individually tested by mating with homozygous mld/mld animals. Those that generated no shaking progeny in at least three litters totalling at least 20 animals were presumed to be homozygous mld'/mld animals and were intercrossed to generate a true breeding mld'/mld' colony. The cross-hatched symbols represent animals carrying the mld' mutation, the filled symbols represent animals carrying the mld' mutation.
animals to generate heterozygous potential recombinant and non-recombinant progeny are indicated. Be detected phenotypically. The genotypes and phenotypes of the represents a null allele at the MBP locus, in the presence of which heterozygotes were mated with 16 of function of the MBP locus on the opposite chromosome can be detected phenotypically. The genotypes and phenotypes of the potential recombinant and non-recombinant progeny are indicated. The phenotype of the potential r/shi recombinant is not known but is assumed to be nonshaking (NS) since in this recombinant the putative r mutation has segregated from the dysfunctional MBP locus associated with mld. At 21 days after birth the progeny were examined for the shaking phenotype characteristic of mld/shi animals. The results were 262 nonshaking (NS), 0 shaking (S). The open symbols represent animals carrying the wild-type allele at the MBP locus. The filled circles represent animals carrying the mld mutation. The stippled symbols represent animals carrying the shi mutation. Crossed to establish a breeding colony of mld'/mld' animals.

The mld' mutation is allelic to the original mld mutation: The mld' animals presumably carry the original mld mutation plus a second mutation that reverses the mld phenotype. The second mutation could be located either at the MBP locus itself, affecting the mld mutation directly (allelic reversion), or at some other site in the genome, affecting the mld phenotype indirectly (second site suppression). These two possibilities can be distinguished by determining if mld' is allelic to mld. This was accomplished as shown in Figure 3. Homozygous mld'/mld' mice were crossed with wild-type mice to generate heterozygous mld'/+ mice. If mld' is not allelic to mld recombination between the two mutations may occur at this stage. The heterozygous animals were crossed with shi/shi mice, shi is used in this cross as a nonreverting, null genetic background on which to assess the function of the MBP locus. The nonrecombinant progeny of this cross are either mld'/shi or +/shi, neither of which shake. However, if there is recombination between mld' and mld some progeny will have lost the mld' mutation but retained the mld mutation and will be mld/shi, which do shake. Thus, if shaking progeny are produced one can conclude that mld' and mld have segregated independently and thus are nonallelic. The percentage of shaking progeny will be proportional to the genetic distance between the two mutations. If no shaking progeny are produced one can conclude that mld' and mld are alleles. We have examined 262 progeny of this cross without finding a single shaking animal. From these results we conclude that mld' and mld are allelic, at a genetic resolution of <1 cM. This means that the mld' mutation may directly affect one or both of the MBP gene copies at the mld locus.

**mld' does not contain the mld-specific inversion junctions**: To examine the structure of the MBP locus in mld' we performed restriction analysis of MBP gene sequences in isolated genomic DNA from wild-type, mld/mld and mld'/mld' animals. Previous studies revealed a partial inversion in the upstream MBP gene copy in mld/mld, the junctions of which create two mld-specific restriction fragments. We have examined restriction digests of mld'/mld' DNA for these two fragments. DNA was isolated from five animals of each genotype, digested with BglII and subjected to agarose gel electrophoresis and Southern blotting. The blots were hybridized with a 4.5-kb BamHI probe, derived from intron 2 in the MBP gene, which spans the mld inversion breakpoint, as diagrammed in Figure 1. The results are shown in Figure 4. Digestion with BglII and hybridization with the 4.5 BamHI probe reveals a 9.3-kb fragment present in all three genotypes. This fragment is derived from approximately 12 to 22 kb in the MBP gene in wild type and from the corresponding region in the intact downstream MBP gene copy in mld. Its presence in mld' provides evidence for at least one intact MBP gene in the revertant. In addition to the 9.3-kb fragment, mld contains fragments of approximately 8 kb and 6 kb. These fragments are derived from the new junctions created at the 3' and 5' ends, respectively, of the inversion in the upstream gene copy in mld. The absence of these fragments in mld' means that this rearrangement has been eliminated in the mld' revertant. This indicates that the reversion affects the MBP locus directly and is consistent with the genetic data indicating that mld' is allelic to mld.

**mld' contains a single intact MBP gene**: There are several possible explanations for the absence of mld-specific inversion junction fragments in mld'. The entire rearranged upstream MBP gene copy in mld may be deleted in mld', leaving the downstream MBP gene intact. The 5' end of the upstream gene may recombine with the 3' end of the downstream gene to reconstitute a single intact gene copy. A portion of the rearranged MBP gene copy, including both the 5' and 3' junctions of the inversion, may be deleted.
in mld', leaving a fragment of the upstream MBP gene copy and the entire downstream MBP gene copy. The inverted region in the upstream MBP gene copy in mld may be reinverted in mld' reconstituting a second intact MBP gene copy. The first two possibilities result in a single copy of the MBP gene in mld', while the last two result in either two copies of the entire gene or one complete copy plus a partial copy of the MBP gene.

To distinguish among these possibilities we determined the MBP gene copy number in DNA from wild type, mld/mld and mld'/mld' animals. This was accomplished by quantitating the levels of MBP-specific restriction fragments from the three genotypes on the blot shown in Figure 4, and on a second blot (not shown) containing additional DNA samples. To normalize for variations in DNA loading, efficiency of transfer and hybridization in different lanes the major intact MBP gene is present in one intact copy per haploid genome. To test the hypothesis that the single MBP gene in mld' contains a recombinant MBP gene derived from

\[ \text{mld'} \] Revertant Mouse

**Figure 4.**—Restriction analysis of MBP gene sequences in wild-type, mld and mld' DNA. DNA from three different animals of each genotype was isolated, digested with BgIII restriction enzyme, subjected to agarose gel electrophoresis, transferred to nylon membrane and hybridized with the 4.5-kb BamHI probe shown in Figure 1. Lanes 1–3 contain wild type DNA, lanes 4–6 contain mld/mld DNA and lanes 7–9 contain mld'/mld' DNA. The positions of the 9.3, 8 and 6 kb fragments are indicated. In addition, several faint non-MBP bands are labeled nonspecifically. The intensities of these bands provide an internal control to normalize for interlane variations in DNA loading, transfer efficiency or hybridization.

The simplest explanation for these results is that mld' contains a recombinant MBP gene derived from

\[ \text{mld'} \] Revertant Mouse

grated number of counts in the 9.3-kb band in each lane was normalized to the mean number of counts in the band over the whole blot. When this was done the gene copy number per haploid genome was calculated to be 1.04 ± 0.35 for wild-type DNA (n = 6), 0.83 ± 0.19 for mld DNA (n = 6) and 1.13 ± 0.38 for mld' DNA (n = 6). Analysis of the variance, assuming a gene copy number of one for each of the genotypes, gives F P = 0.2880 > 0.05, which is strong evidence for a gene copy number of one in mld’. A similar analysis (data not shown) was performed using a probe specific for the promoter region of the MBP gene. This also indicated one MBP gene copy in mld'. It is a reasonable extrapolation from these results that the entire MBP gene is present in one intact copy per haploid genome in mld' DNA.

**mld' contains a recombinant MBP gene:** There are several possible ways of deriving the single MBP gene in mld' from the complex MBP locus in mld. These include: deletion of the entire upstream gene leaving the downstream gene intact; deletion of the entire downstream gene combined with rearrangement of the upstream gene to reconstitute an intact MBP gene; recombination between the 5’ end of the upstream gene and the 3’ end of the downstream gene to generate a recombinant MBP gene. In order to distinguish among these possibilities we have used the polymerase chain reaction to assay the junctions designated AA’, CD, B’X, X’A’ in Figure 1. Oligonucleotides flanking the various junctions were synthesized based on published sequence data (OKANO et al. 1988, 1991). The AA’ oligonucleotide pair should generate a reaction product of 375 bp. The CD oligonucleotide pair should generate a reaction product of 270 bp. The B’X oligonucleotide pair should generate a reaction product of 334 bp. The X’A’ oligonucleotide pair should generate a reaction product of 299 bp. DNA samples from several different animals of each genotype were subjected to polymerase chain reaction with appropriate pairs of oligonucleotides and the reaction products were analyzed by gel electrophoresis. The results are shown in Figure 5. Wild-type DNA generates appropriately sized reaction products with the AA’ primer pair but not with the CD, B’X and X’A’ primer pairs. mld DNA generates appropriately sized reaction products with all four primer pairs. mld' DNA generates appropriately sized reaction products with the AA’ primer pair but not with the CD, B’X and X’A’ primer pairs. This means that the single MBP gene in mld' contains the AA’ junction from the 5’ flanking region of the upstream MBP gene in mld but lacks the CD, B’X and X’A’ junctions contained within the body of the upstream gene.

The simplest explanation for these results is that mld' contains a recombinant MBP gene derived from
Figure 5.—Polymerase chain reaction analysis of wild-type, mld and mld' DNA. DNA from several animals of each genotype was subjected to the polymerase chain reaction with various different pairs of oligonucleotide primers. The reaction products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. Panel A shows the reaction products obtained with primers spanning the AA' junction shown in Figure 1. The size of the major product indicated by the arrow is 375 bp. Panel B shows the reaction products obtained with primers spanning the CD junction. The size of the major product is 270 bp. Panel C shows the reaction products obtained with primers spanning the B'X junction. The size of the major product is 334 bp. Panel D shows the reaction products obtained with primers spanning the X'A' junction. The size of the major product is 299 bp. In each panel the first 4 lanes contain wild type DNA, the next five contain mld/mld DNA, the next five contain mld'/mld' DNA, and the last lane contains pBR322 DNA digested with MspI. The sizes of the MspI fragments are: 622, 527, 404, 242, 238, 217, 201, 190, 180, 160, 147, 123, 110, 90, 76, 67, 34, 26, 15 and 9 bp.

The 5' end of the upstream mld gene and the 3' end of the downstream mld gene. This would require a single recombination event between the region from AA' to CD in the upstream MBP gene and the region from X'A' to CC' in the downstream MBP gene. The result of such a recombination event would be to reconstitute an intact recombinant MBP gene from the 5' end of the upstream gene and the 3' end of the downstream gene, effectively eliminating the intervening DNA, including junctions CD, B'X and X'A'.

An alternative explanation that is consistent with the data but seems less likely is that the single MBP gene in mld' is derived exclusively from the upstream MBP gene copy in mld. This would require at least four separate DNA rearrangements: (1) deletion of the downstream MBP gene copy, (2) deletion of the extraneous DNA segment inserted between the upstream and downstream MBP gene copies (B'X-X'A' in Figure 1), (3) reversion of the inverted segment in the upstream MBP gene copy, and (4) deletion of the duplicated region flanking the inverted segment in the upstream MBP gene copy (BB'-CC' in Figure 1). This series of multiple DNA rearrangements remains a formal possibility. Indeed, a similar series of DNA rearrangements must have occurred to generate
The original mld mutation. However, we feel that it is much more likely that the single MBP gene in mld' represents a recombinant gene derived from the 5' end of the upstream mld gene and the 3' end of the downstream mld gene.

The recombinant MBP gene expression in mld' is functional: The mld'-mutation cures the hypomyelination phenotype. Since the hypomyelination phenotype in mld is attributable to loss of function at the MBP locus, amelioration of the phenotype in mld' implies restoration of function at the MBP locus. To test this we analyzed MBP gene expression in wild-type, mld/mld and mld'/mld' brains at different times during development by SDS-PAGE/immunoblotting. The results are shown in Figure 6. In mld/mld (not shown) the level of MBP was below the limits of detection at every age. In wild type and mld'/mld' MBP was first detected at 15 days of age and increased thereafter. The levels of the four major forms of MBP, with molecular masses of 21.5, 18.5, 17 and 14 kD, were comparable in wild type and mld'/mld' at each age. The quantitative differences in staining between wild type and mld'/mld' are due to differences in amount of protein loaded on the gel. These results indicate that developmental regulation, level of expression, and pattern of post-transcriptional processing of the recombinant MBP gene in mld'/mld' are comparable to the wild-type MBP gene.

DISCUSSION

In this paper we describe a new mouse mutation, mld', which arose in a colony of mld mice and which cures the mld phenotype. We present genetic and molecular evidence that the mld' mutation is the result of recombination of the 5' end of the upstream rearranged MBP gene with the 3' end of the downstream MBP gene copy in mld. This reconstitutes a single functional recombinant MBP gene in mld' and eliminates approximately 50 kb of the MBP locus in mld, including the various duplications, inversions, insertions and rearrangements that disrupt MBP gene expression.

There are several possible mechanisms that could generate the recombinant MBP gene in mld'. (1) Since the region between junctions AA' and CD in the upstream gene copy and between junctions X'A' and CC' in the downstream gene copy in mld are homologous, a single homologous recombination event within this region could generate the recombinant gene. If the recombination event was reciprocal the region between the crossover points would form a circular recombination product which would presumably be lost. (2) Since the MBP gene is believed to contain Z-DNA which promotes nonhomologous recombination (Molineaux et al. 1986), a nonhomologous recombination event between the region from the AA' to CD junctions in the downstream gene and the X'A' to CC' junctions in the downstream gene could also generate the recombinant MBP gene. This could result in either duplication or deletion of DNA in the region of the recombination break points and elimination of the intervening genetic material as a circular recombination product. (3) A large deletion from a point downstream of the AA' junction in the upstream gene to a point upstream of the BglII site at approximately 12 kb in the downstream gene in mld could generate the recombinant MBP gene in mld'. This is formally analogous to the previous mechanism and could result in either duplication or deletion of genetic material flanking the deletion breakpoints. (4) A gene conversion event between the rearranged upstream gene copy in mld and the intact downstream gene on the same or a different chromosome could generate the recombinant MBP gene in mld'. The upstream boundary of such a conversion event would be downstream of the AA' junction in mld. These possibilities cannot be distinguished on the basis of the results presented here. However, a more detailed structural analysis of the recombinant MBP gene in mld' might help to discriminate among them.
more reasonable comparison may be to the excision of endogenous proviruses through recombination between long terminal repeat sequences which occurs at frequencies of $3-4 \times 10^{-6}$ (Frankel et al. 1990; Copeland, Hutchinson and Jenkins 1983). Since the mld" revertant apparently arose through a similar recombination event the germ line reversion frequency at the mld locus may be of a similar order of magnitude.

It is possible that the MBP-positive "revertant" oligodendrocytes which appear at a significant frequency (2–5%) in mld CNS tissue (Shen et al. 1985; Akowitz et al. 1987) represent somatic cells which have undergone recombination events similar to that which occurred in the germline of the mld" mouse. If this were the case it would imply a higher frequency of recombination in somatic cells compared to germline cells. This could reflect the fact that the MBP locus is not expressed in cells other than oligodendrocytes in the CNS and Schwann cells in the PNS. In other systems active transcription appears to stimulate recombination. For example, recombination leading to T cell receptor gene (Ferrier et al. 1990) or immunoglobulin gene (Radcliffe et al. 1990) rearrangement occurs at higher frequencies in lymphoid tissues in which the genes are actively transcribed than in other tissues where they are not expressed. Thus, it is conceivable that recombination within the MBP locus in mld is stimulated when the gene is actively transcribed in oligodendrocytes, leading to a higher reversion frequency in these cells compared to the germline. However, this hypothesis is difficult to test directly because oligodendrocytes are generally postmitotic cells and thus cannot be easily expanded to generate sufficient DNA for analysis.

The question of which aspect of the complex genetic lesion in mld is responsible for the mutant phenotype remains open. The recombinant MBP gene in mld" is indistinguishable, by the techniques used in this study, from the wild-type MBP gene. No trace of the original mld genetic lesion was detected in mld" DNA. The fact that the recombinant MBP gene in mld" is functional implies that the 5' end of the upstream MBP gene and the 3' end of the downstream MBP gene in mld, which make up the recombinant gene, are not intrinsically defective. However, further conclusions concerning the reasons for dysfunctionality of the MBP locus in mld must await isolation of partial revertants in which some parts of the genetic lesion are affected while others remain unaffected.

This work was supported by the following grants: National Institutes of Health (NIH) NS15190 and HCRAC 6-50994 to J.H.C., NIH NS19943 to E.B., Lepow award to K.A., and Health Center summer internship to L.B. We would like to acknowledge the technical assistance of Christopher Barry in performing the immunoblot analyses, Rich Kelly in quantitating the Southern blots, Gordon Carmichael in preparing the oligonucleotide primers and Francesco Avossa in statistical analysis.

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Communicating editor: N. A. JENKINS