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Exon skipping of exonuclease 1 in MRL/MpJ mice is caused by a nucleotide substitution of the branchpoint sequence in intron eight

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Abstract

In MRL/MpJ mice, there is a genetic mutation of exonuclease 1 (*Exo1*), in which the exon 9 is sometimes deleted. In the present study, to check the generation of the spliced exons, exon 8-intron 8-exon 9 (*pCX/Ex/EIE/B* and *pCX/Ex/EIE/M*) plasmids were temporally transfected *in vitro* into BALB 3T3 cells, and RT-PCR using appropriate primer pair was carried out 1 day after transfection. In these constructions, *pCX/Ex/EIE/B* was derived from genomic sequence of C57BL/6 mice, and *pCX/Ex/EIE/M* was from MRL/MpJ. A spliced band was detected in *pCX/Ex/EIE/B*, but was present little or very weakly in *pCX/Ex/EIE/M*. Next, the same spliced band was demonstrated in the *pCX/Ex/EIE/M(T)* plasmid, in which the branchpoint sequence (BPS) of *pCX/Ex/EIE/M* including the exon 9 was changed into that of *pCX/Ex/EIE/B*. The splicing did not occur in the *del1/B* mutant, in which 1960 nucleotides of the intron 8 were deleted, whereas it was detected in the *del2/B* plasmid deleted 1036 nucleotides in its middle region. These results suggest that the nucleotide T to A mutation of the BPS in the intron 8 is at least a sufficient for generation of splice variants (*tr-1* and *tr-2 Exo1*).

Key words : branchpoint sequence ; *exonuclease 1*; exon skipping ; MRL mice ; splicing

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Introduction

It has been reported that the MRL/MpJ strain is strikingly unique in its capacity for regenerative wound-healing, as shown by the closure of ear punches and cardiomyocyte regeneration with normal tissue architecture reminiscent of amphibian regeneration as opposed to scarring^{(7), (9)}. It has furthermore been indicated that the development of several autoimmune diseases, such as systemic lupus erythematosus, polyarteritis nodosa, rheumatoid arthritis, Sjögren's syndrome and systemic sclerosis are dependent on the MRL genetic background, but not on the *lpr* allele^{(12), (17)}.

Recently, we found three unique characteristics in the testis of the MRL/MpJ mouse: metaphase-specific apoptosis of meiotic spermatocytes (*msa*)^{(2), (4)}, heat-shock resistance in spermatocytes found in experimental cryptorchidism (*hsr*)^{(1), (3)} and the appearance of oocyte-like cells during postnatal development (unpublished data).

In a previous study, we found that *msa* was mapped to the telomeric region of chromosome 1 and was significantly linked with *Exo1*⁽¹⁰⁾. The mouse *Exo1* gene consists of 14 exons and 13 introns spanning about 40 kb of DNA, possessing a translational start site in the exon 2 and a stop codon in the exon 14. On the *Exo1* cDNA in strain MRL/MpJ, but not in other strains, it was noted that truncated forms (*tr-1 Exo1* and *tr-2 Exo1*) were expressed in all tissues examined. These were shown to be due to the skipping of exon 9 by comparing their nucleotide sequences with normal one. Additionally, in *tr-1 Exo1*, the stop codon (TGA) appeared in exon 10, leading to prediction of truncated protein. To identify the cause of abnormal splicing of *Exo1*, nucleotide sequencing of the 8th intron was performed paying attention to the splicing signals. As a result, nucleotide substitution in

the branchpoint sequence (BPS) was detected, namely, GGGTAA in C57BL/6 was substituted to GGGAAA in MRL/MpJ.

These results revealed that the cause of abnormal splicing of *Exo1* was nucleotide substitution of BPS in the intron 8. To check and confirm the generation of the spliced variants, an expression plasmid containing the nucleotide sequence from the exon 8 to the exon 9 of the *Exo1* gene was constructed and introduced transiently into the mouse 3T3 cell line. In the present study, the results of RT-PCR clearly showed that the mutation of BPS in the intron 8 caused abnormal splicing of *Exo1* gene in the MRL/MpJ mouse.

Materials and Methods

Animals

Male inbred mice, C57BL/6 and MRL/MpJ- +/+ were purchased from an animal breeding company (Japan SLC, Inc., Hamamatsu, Japan) and maintained in our facility with feeding and drinking *ad libitum*. In the experimental animal care and handling, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals, Hokkaido University, Graduate School of Veterinary Medicine."

RT-PCR and nucleotide sequence of *Exo1*

In order to confirm the previous study, total RNA was prepared from the testis, spleen and thymus of C57 BL/6 and MRL mice by acid guanidium thiocyanate-phenol-chloroform extraction (TRIZOL, Life Tech., Gaithersburg, USA) and subjected to reverse transcription (ReverTra Ace, Toyobo, Osaka, Japan), according to the manufacturers' instructions. PCR for *Exo1* was performed using the primer pair, consisting of the forward: 5'-CAGGATG AATATAACGGTGCC-3' and the reverse: 5'-ATTGCCTGGAATCTGAGAGC-3', in which the amplified length was 919 bp in the posi-

tion of 799-1717 (Accession No. AJ 23823).

PCR was carried out on a Biorad PCR thermal cycler (iCycler, Tokyo, Japan) with the cycling sequence of 94°C for 5 min (one cycle), followed by 35 cycles consisting of denaturation at 94°C for 40 sec, primer annealing at 60°C for 30 sec, and extension at 72°C for 150 sec. The PCR mixture and enzymes, in which there was sufficient proofreading ability, were purchased from Takara (EX Taq Polymerase, Tokyo, Japan) or Toyobo (KOD Dash Polymerase, Osaka, Japan). The amplified samples were electrophoresed with 1% agarose gel, stained with ethidium bromide, and finally photographed under an ultraviolet lamp.

For nucleotide sequencing, the amplified products cloned into the TA-vector (*pGEM-T Easy vector*, Promega, Madison, USA) were used with a cycle sequencing kit containing fluorescent terminators employing standard methods (Applied Biosystems, Foster City, USA) and then analyzed on a model 377 automatic sequencer (Applied Biosystems, Foster City, USA).

Construction of plasmids

Genomic DNA was prepared from the spleen of each animal. These tissues were incubated in lysis buffer containing 50 mM Tris-HCl (pH 8.0), 100 mM NaCl, 20 mM EDTA, 1 % sodium dodecyl sulfate and 100 µg/ml proteinase K, overnight at 56°C, and then treated with two phenol extractions and one phenol : chloroform extraction. Finally, genomic DNA was purified by ethanol precipitation and dissolved in TE (10mM Tris-HCl, 1 mM EDTA, pH8.0).

To amplify the exon8-intron8-exon 9 fragment of *Exo1* spanning about 2.6kb in each strain (*Ex/EIE/B* for C57BL/6 and *Ex/EIE/M* for MRL/MpJ), the primer pair (forward : 5'-ATGTACGTCGGTGACTCTGT-3' annealed on the 5' site of exon 8, reverse : 5'-CATTTCT

TGGTCTTTTCAACACAC-3' annealed on the 3' site of exon 9) was constructed according to a previous report (Accession No. AC073663)¹⁰.

The products amplified by PCR was ligated with TA-vector, and the nucleotide sequence was confirmed on the automatic sequencer. To make original expression plasmids, the expression vector, *pCXN 2* (a kind gift from Dr. J. Miyazaki)¹¹ was ligated with either the *Ex/EIE/B* or *Ex/EIE/M* fragment digested by *EcoRI* from *pGEM-T* plasmid.

Construction of *pCX/Ex/EIE* plasmids and their mutants is shown in Figure 1. Three typed mutants were constructed from *pCX/Ex/EIE/B* or *pCX/Ex/EIE/M* plasmids. First, an exchanged mutant was obtained by cutting original plasmids with *XhoI* and *HindIII*, and the fragments were exchanged with each other. In these, the BPS (GGGTAA) originating from B6 mice was changed into GGGAAA (*pCX/Ex/EIE/B(A)*), whereas that (GGGAAA) from MRL mice was changed into GGGTAA (*pCX/Ex/EIE/M(T)*). Second, in deletion mutant 1 (*del 1/B*, *del 1/M*) on the *pCX/Ex/EIE/B* or *pCX/Ex/EIE/M* plasmid, the proximal and middle regions of 1960 nucleotides between *BglII* and *XhoI* in the intron 8 were deleted. Third, in deletion mutant 2 (*del 2/B*) the middle region of 1036 nucleotides between *EcoT 221* and *XhoI* in the intron 8 was deleted from *pCX/Ex/EIE/B*.

Transfection into BALB3T3 cells and RT-PCR

The *pCX/Ex/EIE* plasmids and their mutants from both strains were temporally transfected into BALB3T3 cells by the lipofection method (FuGene 6, Roche Diagnostics, Tokyo, Japan). After 1 day of transfection, RT-PCR was performed using the appropriate primer pair, consisting of the forward: 5'-GGC TTCTGGCGTGTACC-3' (located just before the exon 8) and the reverse : 5'-CATTTCTT GGTCTTTTCAACACAC-3' (located on the

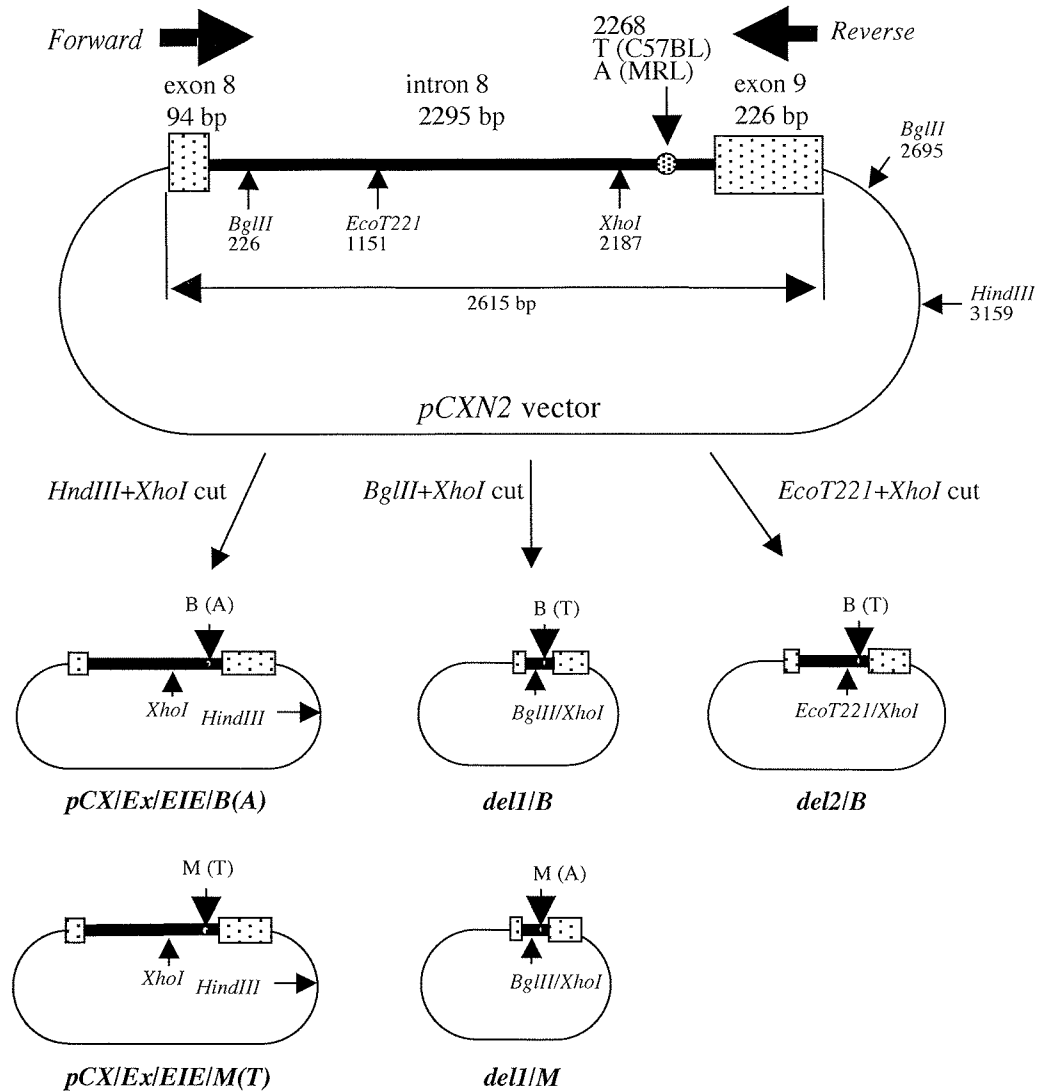


Figure 1. Construction of *pCX/Ex/EIE* and mutant plasmids. The 2268 th nucleotide starting from the 5' site of the exon 8 is inside of the BPS, producing T in *pCX/Ex/EIE/B* derived from C57BL/6 or A in *pCX/Ex/EIE/M* derived from MRL. The numbers presented below each restriction enzyme are the distances from the 5' site of the exon 8. The large arrows showing *Forward* and *Reverse* indicate the primer site used for RT-PCR.

end of the exon 9) primers, in which the length of amplified product was deduced to be 0.3kb in normal splicing of the intron 8. On the other hand, if not abnormally spliced, its length would be 2.6kb in *pCX/Ex/EIE* plasmid, 0.7kb in the *del1* mutant, and 1.6kb in the *del2* mutant, respectively. PCR was carried out with the same protocol described above and the samples were electrophoresed

with 2% agarose gel.

Results

Three types of products were at least detected by RT-PCR for *Exo1* in all tissues of MRL mice, whereas there was just one product in those of C57BL/6 mice (Fig. 2a). Nucleotide sequencing showed that the first type in MRL mice was normal *Exo1*, in which two nu-

cleotides were substituted between C57BL/6 and MRL mice (Fig. 2b). On the other hand, the second and third types had shorter sequences with deletion of 226bp (*tr-1 Exo1*), and with insertion of 205 bp and deletion of 21bp (*tr-2 Exo1*), respectively. The deduced amino acids suggested that there was a stop codon in *tr-1 Exo1* by frame shift, whereas *tr-2 Exo1* was in-frame even though different sequence was inserted (Fig. 2c). The comparison of genomic sequence from exon 8 to exon 9 in *Exo1* between C57BL/6 and MRL mice showed 8-nucleotide substitutions and 9-nucleotide deletions of MRL mice, in which BPS was changed from GGGTAA in C57BL/6 into GGGAAA in MRL mice (Fig. 2d). These results were corresponded with the previous report¹⁰⁾.

In the experiments using the original expression plasmids, a spliced band spanning about 0.3kb could be detected in *pCX/Ex/EIE/B* derived from C57BL/6, but it could not be found or was very weak in *pCX/Ex/EIE/M*

isolated from MRL (Fig. 3). Next, the same spliced band was demonstrated in the *pCX/Ex/EIE/M(T)* plasmid, in which the BPS including the exon 9 was changed from *pCX/Ex/EIE/M* type to *pCX/Ex/EIE/B* type. On the other hand, *pCX/Ex/EIE/B(A)* did not show any spliced band. These results suggested that the nucleotide T to A mutation of the BPS in the intron 8 was at least sufficient for generation of spliced variants *tr-1* and *tr-2 Exo1*.

Splicing expecting 0.7 kb band did not occur in either the *del1/B* or *del1/M* mutated plasmid, in which the 1960 nucleotides of the intron 8 were deleted (Fig. 4). In the *del 2/B* mutant, in which the 1036 nucleotides of the intron 8 were deleted, a spliced band was detected as a product with the same 0.3kb-length as in the original *pCX/Ex/EIE/B* plasmid. These results revealed the possibility that other sequence elements located in the proximal region of the intron 8 than the BPS and polypyrimidine tract may be required for normal splicing.

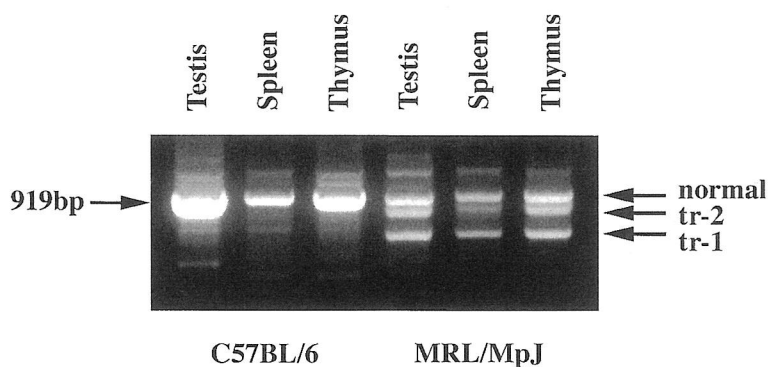


Figure 2 a

Figure 2 . Comparison of partial nucleotide/amino acid sequence of *Exo1* between C57BL/6 and MRL mice. a : Expression of *Exo1* by RT-PCR in various tissues. Primer pair used is mid region of *Exo1* cDNA as described in materials and methods. Three type of products are detected in MRL mice, but not in C57BL/6 mice. b : Nucleotide sequence of the products amplified in Fig. 2a. Forward and reverse primer sequences are underlined. Identical nucleotides are indicated by dots (.), and the numbers with each line indicate the nucleotide position. Nucleotide deletion shown by dashed lines is detected in *tr-1* and *tr-2 Exo1*. Additionally, different sequence is inserted in *tr-2 Exo1*. c : Putative amino acid sequence in C57BL/6 and MRL mice. Stop codon indicated by asterisks is present in *tr-1 Exo1*. d : Genomic sequence from exon 8 into exon 9 in C57BL/6 and MRL mice. Underlines indicate exon 8 and 9. BPSs are shown by outlined box.

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C57BL/6 CAGGATGAAT ATAACGGTGC CCGAGGATTA CATCACAGGA TTTATTCGTG CCAACAATAC TTTCCTCTAC CAGCTCGTGT TCGACCCCAT CCAAAGGAAG 100
MRL-nor .....
MRL-tr1 .....
MRL-tr2 .....

CTGGTCCCTC TGAATGCCTA CCGAGATGAC GTTAATCCCG AAACACTGAC TTACGCTGGG CAGTACGTCG GTGACTCTGT AGCTCTTCAG ATCGCCCTTG 200
.....
.....

GAANTAGAGA TGTAATACT TTTGAACAGA TTGATGACTA CAGTCCAGAC ACCATGCCAG CCCACTCAAG AAGCCACAGC TGGAATGAGA AAGCAGGTCA 300
.....
.....

GAAACCACCT GGTACCAACA GCATTTGGCA CAAGAATTAT TGTCTAGAC TTGAGGTGAA CAGTGTCTCC CACGCTCCTC AACTGAAGGA AAAGCCCAAGC 400
.....
.....
G.....
CAAGAGCTTT GAGGCGGGTC TTTCCCTGCT CAGCAGCCGT GAAGTACTTC CGTACTCAGC GCTATGCAGA GCGTGCATGA AAATGACTTC ACCTGGTGTG

ACTTTGGGCC TTAACAAGT GATTAGTACT AAAGGGTTAA ATCTTCCCAG GAAGTCTTGT GTGTTGAAAA GACCAAGAAA TGAAGCGCTG GCTGAAGATG 500
.....
.....
GCAGGCGTCT GTAATCCAG GACTGGGAC GCTGAGACAG GAGAATCGCC ACAAGTTCAA -----

ACCTGTGAG CCAATATTCC TCAGTTTCAA AGAAGATCAA GGAATAATGGC TGTGGGGATG GCACATCACC TAACTCTTCT AAAATGTCCA AGTCCCTGCC 600
.....
.....

CGATTCTGGG ACTGCTCACA AGACAGATGC ACACACCCCG TCTAAGATGA GGAATAAATT TGCAACGTTT TTACAGAGGA GGAATGAAGA AAGCGGTGCA 700
.....
.....

GTCTGGTTC CAGGGACCAG AAGCAGGTTT TTTTGCAGTT CTCAGGATTT TGACAATTC ATACCAAAAA AAGAAAAGCGG CCAGCCTCTA AATGAAACTG 800
.....
.....

TGCCACTGG CAAAGCCACC ACCAGCCTCC TCGGGCACT GGACTGTCCA GACACGGAAG GCCACAAGCC GGTGTGATGCA AATGGGACGC ACAATCTGAG 900
.....
.....
.....T.....
.....T.....
.....T.....

CTCTCAGATT CCAGGCAAT 919
.....
.....

```

Figure 2 b

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C57BL/6 RMNITVPEDY ITGFIRANNT FLYQLVFDPI QRKLVPLNAY GDDVNPETLT YAGQYVGDVS ALQIALGNRD VNTFEQIDDY 80
MRL-nor .....
MRL-tr1 .....
MRL-tr2 .....

SPDTMPAHSR SHSWNEKAGQ KPPGTNSIWH KNYCPRLEVN SVSHAPQLKE KPSTLGLKQV ISTKGLNLPR KSCVLKRPRN 160
.....
.....A.....
.....
.....VTFNN CYISVFEFCL KSFEAGLSLL SSREVLPIYA LCRACMKMTS PGVAGVCNPR TGDAETGESP QVQ-----

EALAEDDLLS QYSSVSKKIK ENGCGDTSP NSSKMSKSCF DSGTAHKTD HTPSKMRNKF ATFLQRRNEE SGAVVVPGTR 240
.....
.....
KRWLKMTCA SIRQFQRRSR KMAVGMHHL TLLKCFSPAP ILGLLTRQMH TPLRL*GINL QRSYRGGMKK AVQSWFQGPPE

SRFFCSSQDF DNFIPKKEG QPLNETVATG KATTSLLGAL DCPDTEGHKP VDANGTHNLS SQIPGN 306
.....
.....M.....
AGFFAVLRIL TISYQKKKAA SL*MKLWPLA KPPPASSGHW TVQTRKATSR LMQMGCCTI*A LRFQA
.....
.....M.....

```

Figure 2 c

C57BL/6
MRL

ATGTACGTCGGTGACTCTGTAGCTCTTCAGATCGCCCTTGGAAATAGAGATGTAAACTTTTGAACAGATTGATGACTACAGTCCAGACACCATGGTAA 100

 CATTTAACAACCTGTACATAAGCGTGTGAGTTTGTCTCAAGAGCCTTTGAGGCGGGTCTTTCCCTTGTCTCAGCAGCCGTGAAGTACTTCCGTACTCAGC 200

 GCTATGCAGAGCGTGCATGAAAATGACTTCACCTGGTGTGGCAGGCGTCTGTAATCCCAGGACTGGGACGCTGAGACAGGAGAATCGCCACAAGTTCAA 300

 GGTAGCCTGGGCTATACAGTGAATCTTGTCTTTCTGGTTTGTGTTTAAAGACAGGTTTTCACCTGTAAGTCTAGCTGTCAAGAACTCATTTGTGTAGA 400

 CCAAGATGGCCCTCGAACFCGTGGAGATTGTCTGACTCTGCTCCCTGGGTACTGGGCATGTGCCACCACATCTAGCCAATTACTTTTAAAAAATGATTAA 500
TG.....
 CTTGATCCGAGAGAAATATCTAATTAATATAGGTGATTATAATACTAAAAACTGATTACATGATATTTTAAAGACCAGCATATGATACATTCAGCAC 600
G.....
 CTTTGTCTCATCTTACGTCATTACAGACTTTGGCACTTTGGCATTGTTGAAAACCCAAAACATGGAGTACCAGCTGCTTAGGCTAATCCGTCAAATACTC 700

 AGTGAGCTGCCCTCCCTTGTGTGTGAACCTTTCACCTGCTCTCTTTGTTGTATACCCTCTGGCAGCGTGTGCCACCTTTCAGGTTATGTTTATGTCT 800

 TCAATAAGTCTTTTAGTACTGTAATTTCTTTTGTCTTTGAGAACGCCATATATGTATAGTGTAGGCCATTTATGCACAGAACCTGAGTTAGTTTAT 900

 CTTATGTTGGGAATCATTACCGAGATGAAAGTCTGGAACACTGGCTGCTGTGTCATTTACACATCTGAATTAGCAATTCAGTAATTTTACCAGGAGTG 1000

 ATGCTGAAGGTGTTTGTCTTAAACCCACAATTTGTCGCACCGACCTGTAGGTTCTGAGCTGCTAAGGTGATCCTGATTACAGAAACCAATCTATGCCT 1100

 CATCCTTTTATAGGAAACTATACAGCAACGATATGATCTAAAGTGTGGCTTCCCTGGGTTGTTTGGTTTTTTGGGTTTGTTCGGTTTTTGT 1200

 TTTTGGTCTTTTTTCTTTTGCCTATTTCTTTTTCTCAGGAAATGCATCTGTGGATTAAATCTGAGAGCCATGCAGCGGCTGAGGCTGTTTGTGTTC 1300

 TCAAGCCTTAAGTTAACCTTCCCTGGCAATTCAGAGCCTGTTTTGAATGCTGTCTGTTTTTTTTTCCCTTTTACTTTCAGCAAAAAGACTTTTTTTT 1400

 TTTTTTTTGGTTTTAGTACATTTGAGAATGGTTATGTGACAGTCATACTTCGAGATTTTCTGTTTTGTTCTTGTTTTTTGGAGACAGATCTCTCCCA 1500

 GCTCTGGCCGTTCTGGAACCTCACTGTGTAGAACCGCTGCCTCTCAAGTGTGGGTTAAATGCGTGTGCCACCACAGCCCAATTTTCTGTG 1600
A.....T.....
 TCTTCACTTTTTGGTCTTGGCTGCCTTTGTCTATTGTAATAGCGTTACCGATGTTTCTAGCGGTCTTCTGTAACAGTTGTAATCATAAATCTATCAGTGA 1700

 GACTGTTACCTTGGGTTAGCTGGCGTCAACCTTTCTCCAAGTTTTATGCACGATTCAGTGTGAGCCAGAACAGCTTTCTCTTTTCCAGCATGGT 1800

 ACCAAGTGAATCCGATACAACAACAAGAATTTCTAACCTAGGTTGTTCTGTTTGAACGTAACAATTTTTATCCAGGCTTGCAGCTTATGGTTATA 1900

 ATCCAGCATGTGGGAGACTGAGGAGGAGATAAATTCAGCTAGCTAGACTACAGGAGACCTGTCTCAAAATAAATTTAAATTAACACATTTCA 2000
T.....
 GATTGGAGAGATGGCTCAGCGGTTAAGAGCACTGACTGCTCTCCAGAAGAAAGCAAACGTTTATTTAGAGCAGAGACCATGAGATCCCTTCCCTCTCT 2100

 CTCTCTCTCTCTCTCTCCACCAGGCCCCACATGTGCTAGGCAAGTACTGCGCCCTGAGCCATTTTTAATTCAAAAATAAATAAATAGTGTCTCAGAAGT 2200

 CACCTAGACTAGTACTAGTCTGAGCTCACTGTAACCTAAGCAGCCTTTGAATTTTCAATCCCTGCCTCACCAGGCCAGCTTCGAGAGTTTTTCTCTT 2300

 GCACACTAATGAACATCATAAAGCAGACACAGGAGAAATGGTCACGCTCTCTGTTCCGGTAACTCTTTTCTTTTCTTTCTTAGCCAGCCCACTC 2400
GGTAACTTTTCTTTTCTTTCTTTCTTAGCCAGCCCACTC
A.....
 AAGAAGCCACAGCTGGAATGAGAAAAGCAGGTCAGAAAACCTGGTACCAACAGCATTTGGCACAAGAATTATGTCCTAGACTTGAAGTGAACAGTGTGC 2500
G.....
 TCCCAGCTCTCTCAACTGAAGAAAAGCCAAGCACTTTGGGCTTAAACAAGTGATTAGTACTAAAGGTTAAATCTTCCCAGGAAGTCTTGTGTGTGA 2600

 AAAGACCAAGAAATG

Figure 2 d

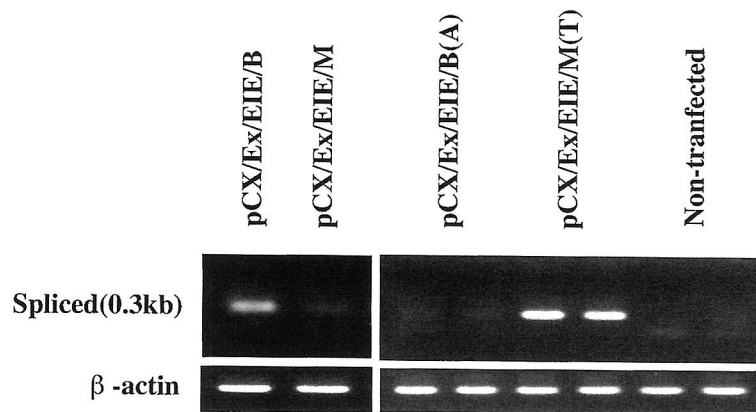


Figure 3 . *In vitro* splicing assay with original and exchanged plasmids. The deduced 0.3kb-length bands are detected in the cells into which *pCX/Ex/EIE/B* and *pCX/Ex/EIE/M(T)* plasmids were introduced by RT-PCR. For internal control, β -actin was used.

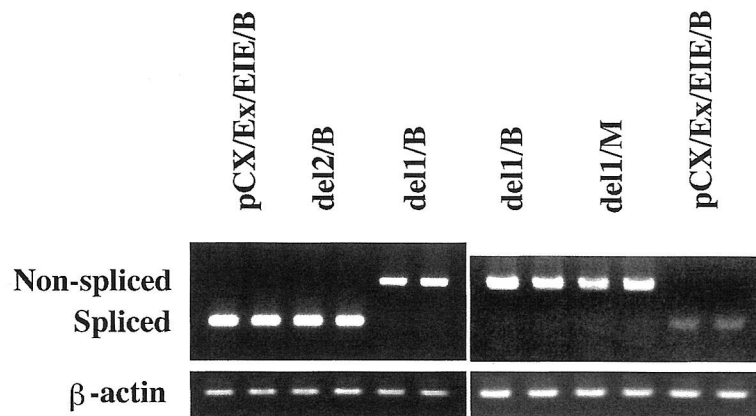


Figure 4 . *In vitro* splicing assay with *del1* and *del2* mutant plasmids. The spliced 0.3kb-length bands are detected in *pCX/Ex/EIE/B* and *del2/B* plasmids; however, in *del1/B* and *del1/M* mutants non-spliced 0.7 kb-length bands are detected. For internal control, β -actin was used.

Discussion

Previous work has shown that the high fidelity of splice-site recognition in pre-mRNA splicing involves specific networks of RNA-protein, protein-protein, and RNA-RNA interactions^{13), 15, 16)}. These interactions are established on the three intronic sequence elements required for splicing: the 5' splice site, the BPS, and the polypyrimidine tract/AG dinucleotide at the 3' splice site^{5), 14)}. We organized this study to clarify why the exon 9 is abnormally deleted during pre-mRNA splicing of *Exo1* in MRL mice.

The present study and previously sequenced data showed that the above three signals were undoubtedly functional in the *pCX/Ex/EIE/B* plasmid¹⁰⁾. Additionally, the results obtained from the exchanged mutants composing *pCX/Ex/EIE/B(A)* and *pCX/Ex/EIE/M(T)* plasmids suggested the importance of the BPS for intron splicing. We did not check mutants other than *del1* and *del2*; however, the first determination of whether the intron 8 was spliced was clearly dominated by the sequence in BPS. The splice reaction takes place in two catalytic steps¹⁵⁾. In the first reaction, the 2' hydroxyl of the conserved

adenosine residue within the BPS attacks the phosphate at the 5' splice site, producing a free 5' exon and a lariat intermediate. In the second reaction, the 3' hydroxyl of the 5' exon attacks the phosphate at the 3' splice site, generating spliced exons and a lariat intron. In the present study, it was possible that the triple adenosine repeat observed in the BPS of MRL mice could not recognize the 5' splice site of intron 8, with the result that the BPS within the intron 9, not the intron 8, attacked the 5' splice site of intron 8. These results suggest that the intron8-exon9-intron 9 complex is spliced all together from the genomic DNA of MRL mice, resulting to produce *tr-1 Exo1* mRNA, in which exon 9 is deleted.

In this study, no intronic splicing occurred on either *del1/B* or *del1/M*. These results revealed the possibility that another still unknown nucleotide sequence influenced the normal pre-mRNA splicing. This important signal might be restricted to the proximal region of the intron 8 with 925 bp between *BglII* and *EcoT221*. However, it was also assumed that instability of a small sized lariat intermediate was caused not to be able to attack the 5' or 3' spliced site. It was outside of the scope of the present study, to determine why *tr-2 Exo1* was produced in MRL mice. However, since in *tr-2 Exo1*, an optional 205 bp sequence, part of the intron 8 directly introduced from the exon 8, was inserted into the deleted region of *tr-1 Exo1*, this could be clarified by the experiment constructing a mutant plasmid throughout the 8-10th exons for investigation of *tr-2 Exo1* production.

Since the abnormality of *Exo1* was associated with mismatch repair for DNA duplication, our results indicated that MRL/MpJ mice are a useful model for investigation of cell proliferation^{6),8)}. According to the results from database searches, incomplete abnormal splicing of *Exo1* in MRL mice produces *tr-1*

protein in which a nuclear localization signal, composing KRPR at 417-420 amino acids is deleted. It is unknown whether or not this truncated protein acts as a dominant negative product. In any case, further investigation is necessary to identify the relationship between the production of mismatched DNA and the activity of *Exo1* in MRL/MpJ mice.

Acknowledgements

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