Comparison of Somatic Mutation Frequency among Immunoglobulin Genes

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Summary

We analyzed the frequency of somatic mutation in immunoglobulin genes from hybridomas that secrete anti-(4-hydroxy-3-nitrophenyl)acetyl (NP) monoclonal antibodies. A high frequency of mutation (3.3–4.4%) was observed in both the rearranged VH186.2 and Vλ1 genes, indicating that somatic mutation occurs with similar frequency in these genes in spite of the absence of an intron enhancer in λ1 chain genes. In contrast to the high frequency in J-C introns, only two nucleotide substitutions occurred at positions −462 and −555 in the 5′ noncoding region in one of the λ1-chain genes and in none of the other three so far studied. Since a similar low frequency of somatic mutation was observed in the 5′ noncoding region of inactive λ2-chain genes rendered inactive because of incorrect rearrangement, this region may not be a target or alternatively, may be protected from the mutator system. We observed a low frequency of nucleotide substitution in unrearranged Vλ1 genes (∼1/15 that of rearranged genes). Together with previous results (Azuma T., N. Motoyama, L. Fields, and D. Loh, 1993. *Int. Immunol.* 5:121), these findings suggest that the 5′ noncoding region, which contains the promoter element, provides a signal for the somatic mutator system and that rearrangement, which brings the promoter into close proximity to the enhancer element, should increase mutation efficiency.

The Ig gene family consists of H, κ, and λ chain genes, each of which has a unique structure in terms of the length of the J-C introns, and the number and location of enhancer elements (1–6). H and κ chain genes contain two enhancer elements, one in the J-C intron (intron enhancer) and the other, 3′ to C exons (3′ enhancer), whereas the λ chain gene lacks an intron enhancer (4–6). In a previous paper (7), we showed that a reporter gene, chloramphenicol acetyl transferase (CAT)[1], was recognized as a target by the somatic mutator system when its expression was controlled by the VH promoter and IgH intron enhancer. Although the mechanism of somatic mutation is not yet understood, these results suggest that the promoter and enhancer elements, but not the V-(D)-J exon, are essential for induction of somatic mutation. Since λ chain genes lack the intron enhancer, it is possible that the frequency of somatic mutation is different between H and λ chain genes. Cumano and Rajewsky (8) reported a higher frequency of somatic mutation in the VH gene than in the Vλ1 gene from idiotype suppressed C57BL/6 mice after immunization with NP-chicken gamma globulin (CGG). The question arose as to whether such a skewed expression of somatic mutation was due to a difference in the effectiveness of promoter and enhancer functions or due to the use of these idiotypically suppressed mice. We addressed this question by comparing the DNA sequences of VH-D-JH and Vλ1-Jλ1 genes from the same hybridomas prepared from mice immunized with only NP-CGG.

If the promoter and enhancer play a critical role in expression of somatic mutation, it was expected that these regions would not be a target for somatic mutation. A low frequency of somatic mutation was shown in the promoter region of VH genes, which suggests the importance of its transcription (9–11). To estimate the 5′ boundary for somatic mutation in λ1 chain genes, we first examined its frequency in the promoter region. However, an analysis using Ig genes from Ab-producing hybridoma cells would not be relevant because only genes with a promoter that retains this activity would be selected. Therefore, we analyzed the DNA sequences of the promoter region in λ2 chain genes that were inactive because of incorrect recombination in order to determine whether the lower frequency was related to gene expression.

Finally, we examined DNA sequences of unrearranged Vλ1

[1] *Abbreviations used in this paper:* CAT, chloramphenicol acetyl transferase; CGG, chicken gamma globulin; NP (4-hydroxy-3-nitrophenyl)acetyl.

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genes. It is important to analyze somatic mutation in unrearranged V genes in order to determine whether the promoter element alone, without cooperation of the enhancer, is capable of inducing somatic mutation. Somatic mutation was observed in unrearranged Vλ1 and Vλ2 genes from a myeloma MOPC315 which secretes IgAλ2 myeloma protein (12), although none was observed in unrearranged V genes of other plasmacytomas (13–16). Since these studies were performed using plasmacytomas and since the number of somatic mutation in the rearranged genes used as controls was rather low, we thought it necessary to determine whether somatic mutation occurs in unrearranged genes using hybridomas with a high frequency of somatic mutation in their rearranged genes (17).

Analyses of the frequency and location of somatic mutation are obviously important to further elucidate the somatic mutation mechanism (9, 10, 18–20). In this study, we compared the frequency among Ig genes from hybridomas in which rearranged Vλ1 genes were shown to have a high level of somatic mutation (17).

Materials and Methods

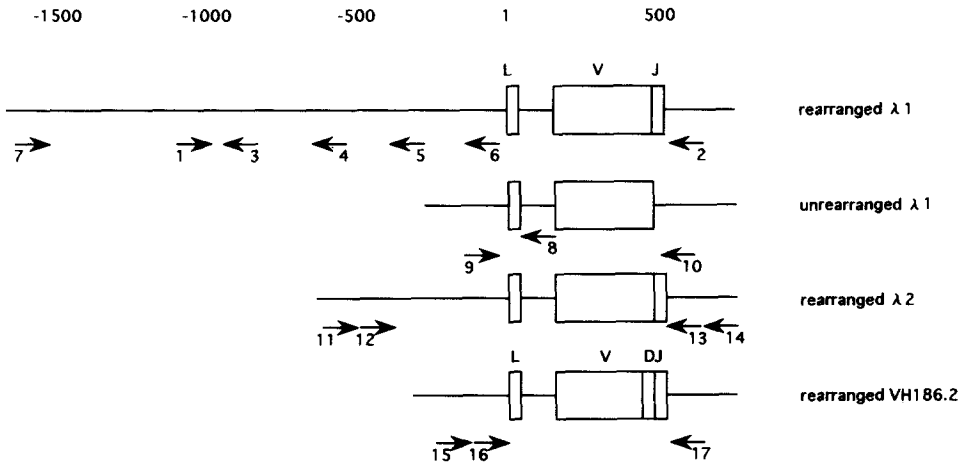
Hybridomas. Hybridomas producing anti-NP mAbs were prepared at 5 wk (5E2, γ2bλ1), 12 wk (C6-8-2, γ1λ1), and 42 wk (E3-19, γ2aλ1; E11-14, γ2aλ1) after immunization of C57BL/6 mice with NP-CGG (17, 21).

PCR, Cloning, and Sequencing. DNA was prepared from liver and hybridoma cells. Genomic DNA was amplified in a thermal cycler (Perkin-Elmer Corp., Norwalk, CT) using a Gene-Amp kit (Cetus, Norwalk, CT). The oligonucleotide primers used for the cloning of Vλ1, Vλ2 and VH186.2 genes are shown in Fig. 1. Since DNA sequences for the 5′ noncoding regions flanking Vλ1 or Vλ2 genes from BALB/c mice had been determined (Motoyama, N., unpublished results), the primers which crosshybridize to C57BL/6 DNA were synthesized. For amplification, either liver or hybridoma DNA (0.1 μg) was mixed with appropriate primers shown in Fig. 1 at concentrations of 0.5 μM. The thermal protocol included 30 cycles of denaturation at 94°C for 1 min, primer annealing at 50°C for 1 min, and extension at 72°C for 2 min. The amplified DNA was digested with EcoRI and subjected to 1% agarose gel electrophoresis. The DNA was purified with silica beads and ligated with pBluescript SKII(+) and the ligation mixture was transfected into *Escherichia coli* XL1-Blue. DNA from putative recombinants was analyzed by restriction enzyme digestion to confirm that a fragment of the appropriate size had been cloned.

Cloned DNA was sequenced by the dideoxy method with primers, as shown in Fig. 1. To minimize cloning artifacts, PCR and cloning were performed twice independently. At least two to six clones from each PCR were subjected to sequencing, but only the consensus nucleotide sequences produced in independent experiments are presented in this paper. In some experiments, DNA sequences were analyzed using an automatic 373A sequencer and a Taq Dye Primer Cycle Sequencing Kit, T3/T7 (Applied Biosystems, Foster City, CA).

Results

Fig. 2 shows the DNA sequences of rearranged VH-D-J genes from hybridomas secreting anti-NP mAbs, E3-19, E11-14, 5E2, and C6-8-2. As has been shown previously (22, 23), the major population of anti-NP Abs bearing λ1 chains from C57BL/6 mice use VH186.2, DFL16, and J2 gene segments. In fact, all four mAbs sequenced were encoded by VH186.2 and J2 gene segments; E3-19, E11-14, and 5E2 use DFL16, and C6-8-2 uses DQ52 (24). The DNA sequences were compared with the germline structure of respective gene segments. 12–29 nucleotide substitutions arising from somatic mutation were observed in VH genes. These occur predominantly in the CDR1 and CDR2. Previously, we examined the DNA



1. 5′-ACAGAATTCTATGCCCACGTTGAAGACAAA-3′
2. 5′-CATGAATTCAGCACCTCAAGTCTTGGAGAG-3′
3. 5′-AAACATAGGGTGACAGAATCT-3′
4. 5′-AGAAGTTAATGTGTATATAGC-3′
5. 5′-TGTTCATCCCAATGGTCAGTT-3′
6. 5′-TACTAAGAACAGAGCTCAGGG-3′
7. 5′-CATGAATTCACTCAATCCCAAACTGGTGGAAGT-3′
8. 5′-ATTGTTGCATACCCACTG-3′
9. 5′-CTGCTGACCAATATTGAAAAG-3′
10. 5′-CACGAATTCGTAGCCACCTGTTAAGAAGA-3′
11. 5′-CCGGAATTCCATTCCATTTCTCAATCATC-3′
12. 5′-GGCGAATTCCATTTCCCAGATTTAGACTC-3′
13. 5′-CATGAATTCTATTAGGAAGAAGCTTTGAAA-3′
14. 5′-CTCGAATTCAGACCCTTGCAAGACAACAAG-3′
15. 5′-CCTGACCCAGATGTCCCTTCTTCTCCAGCAGG-3′
16. 5′-CCCCGAATTCATGGGATGGAGCTGTATCAT-3′
17. 5′-GGGCTCGAGGGTGTCCCTAGTCCTTCATGACC-3′

Figure 1. Schematic presentation of Vλ and VH genes and oligonucleotide primers used for PCR, cloning, and sequencing.

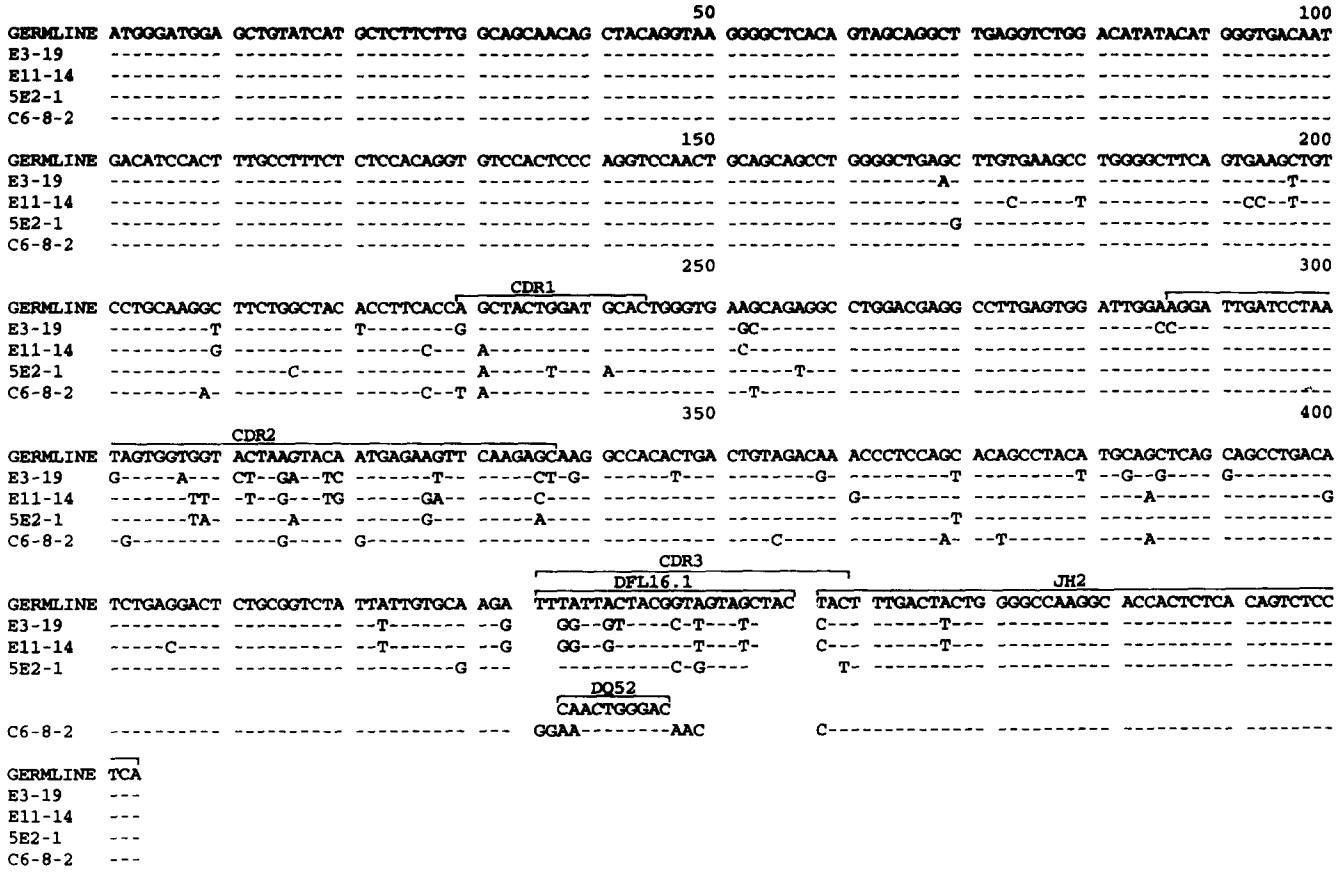
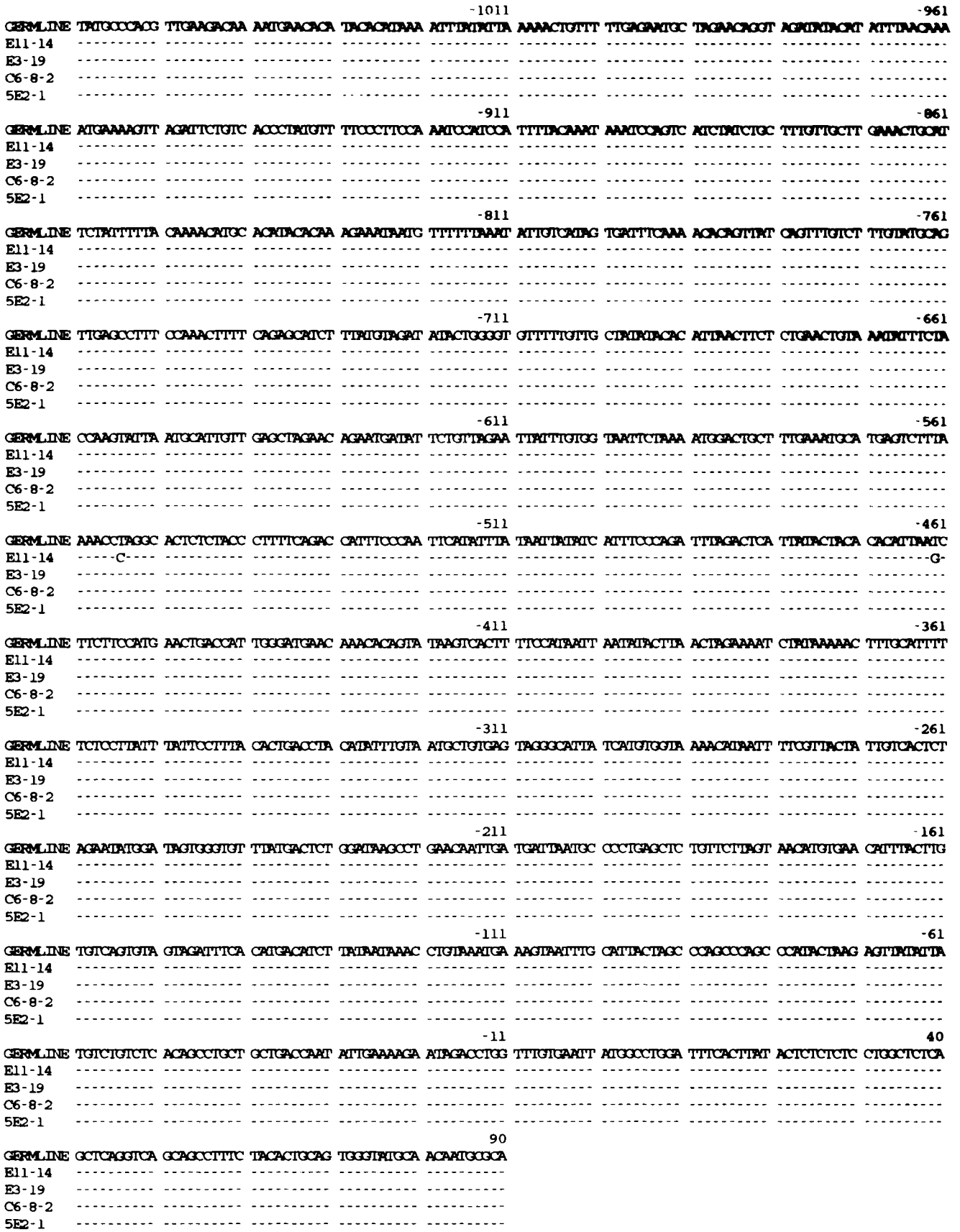


Figure 2. Nucleotide sequences of the Leader-V intron and V-D-J exon expressed in anti-NP mAbs are aligned with the germline sequence of VH186.2 and J2 genes. Since 5E2, E3-19, and E11-14 were encoded by DFL16.1, whereas C6-8-2 was encoded by DQ52, corresponding germline sequences are shown.

sequences of rearranged Vλ1-Jλ1 genes in the same hybridoma used in the present experiment and found 9–24 mutations also predominantly in CDR1 and CDR2 of Vλ1 genes (17). Therefore, the number and the distribution of somatic mutations in VH genes were similar to those in their Vλ1 gene counterparts in the same Ab-producing cells.

Fig. 3 shows the DNA sequences of the 5′ noncoding regions in active λ1 chain genes. In contrast to DNA sequences of the coding region or of the Jλ1-Cλ1 intron (17), only two mutations at position −462 (T to G) and −555 (T to C) from the start codon, ATG, were observed in E11-14. No substitutions, deletions, or additions of nucleotides were detected in the region from −1,059 to 90 of E3-19, 5E2, and C6-8-2. The frequency of somatic mutation was calculated to be 0.04%, which is ∼1/30 of that observed in the Jλ1-Cλ1 noncoding region. Since we prepared hybridomas secreting anti-NP mAbs, the genes encoding these Abs may be positively selected, that is, the genes in which the promoter element loses function by somatic mutation would not be capable of Ab synthesis. Using inactive Vλ2-Jλ2 genes with a frequency of somatic mutation similar to that of active Vλ1-Jλ1 genes (17), we examined whether such mutation occurs in the region 5′ upstream of Vλ2. Nucleotide sequences of inactive Vλ2-Jλ2 genes from E3-19 and E11-14 are compared with those from liver cells in Fig. 4. The sequences of coding regions were in agreement with those reported previously except for the position 444, at which we had assigned T in a previous experiment (17) but found to be C in this experiment. In any case, the sequences of coding regions showed a high frequency of nucleotide substitution. On the other hand, only two nucleotide substitutions were observed in the 5′ noncoding regions at positions −119 (T to C) and −436 (A to C) for E3-19, and −99 (A to G) for E11-14. Although the number of samples analyzed was limited because of the low numbers of rearranged genes in both λ1 and λ2 loci in one cell (one was active and the other inactive) (25), it was evident that somatic mutation occurs less frequently (0.4%) in the 5′ noncoding region of inactive λ2 genes from about −500 bp to the initiation codon, similar to active λ1 genes.

The distribution of somatic mutation in λ1 chain genes is summarized in Fig. 5. The published results are also included in this figure (17). The highest frequency of somatic mutation occurred in the Vλ1-Jλ1 coding region, followed by gradually lower levels along the J-C intron and even into the Cλ1 region. On the other hand, the frequency of so-


-1011 -961
GERMLINE TATGCCCACG TTGAAGACAA AATGAACACA TACACATAAA ATTTATATTA AAAACTGTTT TTGAGAATGC TAGAACAGGT AGATATACAT ATTTAACAAA
E11-14 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E3-19 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C6-8-2 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
5E2-1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
-911 -861
GERMLINE ATGAAAAGTT AGATTCTGTC ACCCTATGTT TTCCCTTCCA AATCCATCCA TTTTACAAAT AAATCCAGTC ATCTATCTGC TTTGTTGCTT GAAACTGCAT
E11-14 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E3-19 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C6-8-2 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
5E2-1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
-811 -761
GERMLINE TCTATTTTTA CAAAACATGC ACATACACAA AGAAATAATG TTTTTTAAAT ATTGTCATAG TGATTTCAAA ACACAGTTAT CAGTTTGTCT TTGTATGCAG
E11-14 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E3-19 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C6-8-2 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
5E2-1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
-711 -661
GERMLINE TTGAGCCTTT CCAAACTTTT CAGAGCATCT TTATGTAGAT ATACTGGGGT GTTTTTGTTG CTATATACAC ATTAACTTCT CTGAACTGTA AAATTTTCTA
E11-14 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E3-19 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C6-8-2 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
5E2-1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
-611 -561
GERMLINE CCAAGTATTA ATGCATTGTT GAGCTAGAAC AGAATGATAT TCTGTTAGAA TTATTTGTGG TAATTCTAAA ATGGACTGCT TTGAAATGCA TGAGTCTTTA
E11-14 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E3-19 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C6-8-2 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
5E2-1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
-511 -461
GERMLINE AAACCTAGGC ACTCTCTACC CTTTTCAGAC CATTTCCCAA TTCATATTTA TAATTATATC ATTTCCCAGA TTTAGACTCA TTATACTACA CACATTAATC
E11-14 -----C---- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------G-
E3-19 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C6-8-2 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
5E2-1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
-411 -361
GERMLINE TTCTTCCATG AACTGACCAT TGGGATGAAC AAACACAGTA TAAGTCACTT TTCCATAATT AATATACTTA ACTAGAAAAT CTATAAAAAC TTTGCATTTT
E11-14 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E3-19 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C6-8-2 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
5E2-1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
-311 -261
GERMLINE TCTCCTTATT TATTCCTTTA CACTGACCTA CATATTTGTA ATGCTGTGAG TAGGGCATTA TCATGTGGTA AAACATAATT TTCGTTACTA TTGTCACTCT
E11-14 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E3-19 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C6-8-2 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
5E2-1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
-211 -161
GERMLINE AGAATATGGA TAGTGGGTGT TTATGACTCT GGATAAGCCT GAACAATTGA TGATTAATGC CCCTGAGCTC TGTTCTTAGT AACATGTGAA CATTTACTTG
E11-14 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E3-19 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C6-8-2 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
5E2-1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
-111 -61
GERMLINE TGTCAGTGTA GTAGATTTCA CATGACATCT TATAATAAAC CTGTAAATGA AAGTAATTTG CATTACTAGC CCAGCCCAGC CCATACTAAG AGTTATATTA
E11-14 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E3-19 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C6-8-2 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
5E2-1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
-11 40
GERMLINE TGTCTGTCTC ACAGCCTGCT GCTGACCAAT ATTGAAAAGA ATAGACCTGG TTTGTGAATT ATGGCCTGGA TTTCACTTAT ACTCTCTCTC CTGGCTCTCA
E11-14 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E3-19 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C6-8-2 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
5E2-1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
90
GERMLINE GCTCAGGTCA GCAGCCTTTC TACACTGCAG TGGGTATGCA ACAATGCGCA
E11-14 ---------- ---------- ---------- ---------- ----------
E3-19 ---------- ---------- ---------- ---------- ----------
C6-8-2 ---------- ---------- ---------- ---------- ----------
5E2-1 ---------- ---------- ---------- ---------- ----------

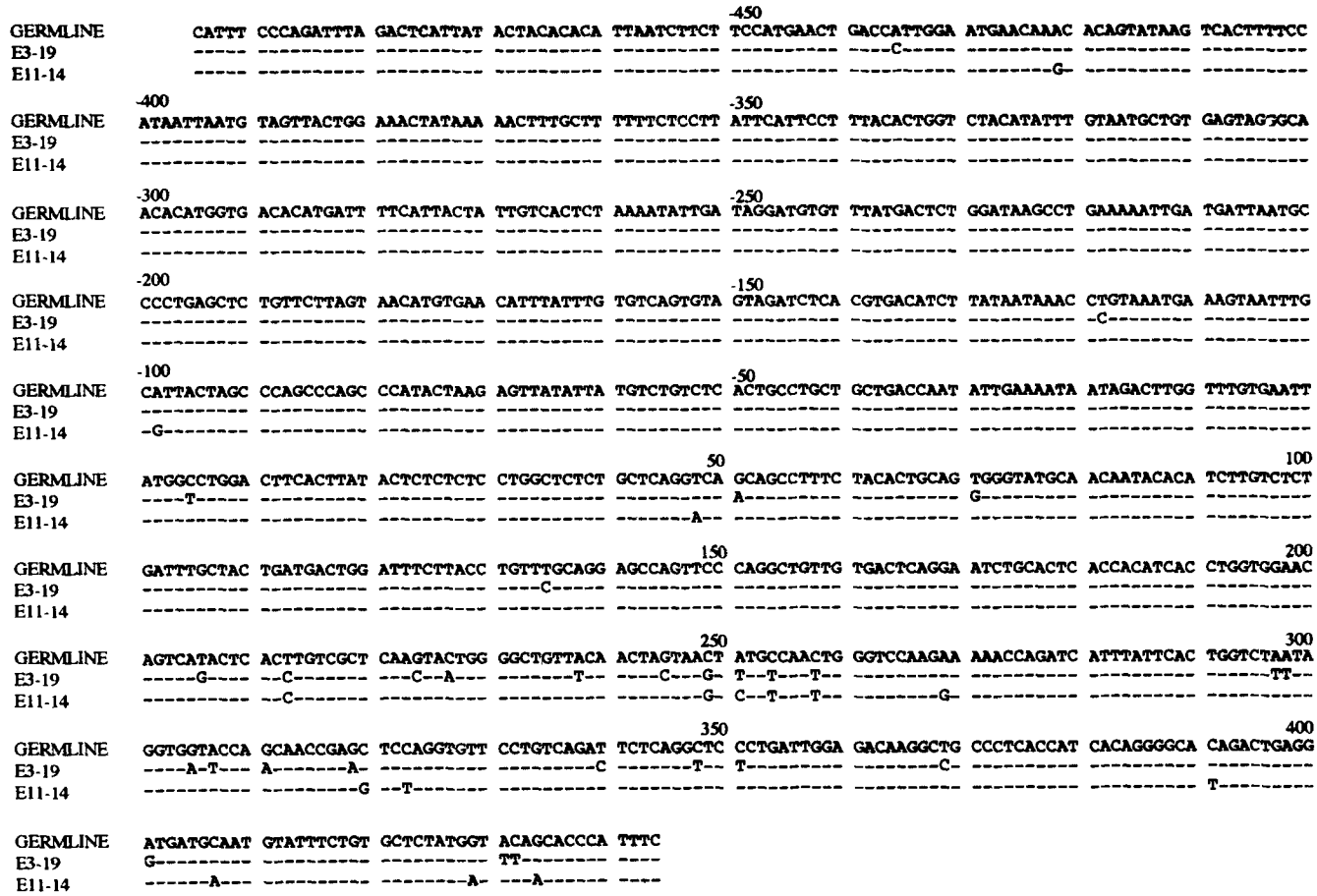


Figure 4. Nucleotide sequences of inactively rearranged Vλ2 genes in hybridomas, E3-19 and E11-14, producing anti-NP mAbs bearing λ1 chains. The germline sequence obtained from liver DNA was compared with those from E3-19 and E11-14. (- - -) Nucleotides identical to the germline sequence.

matic mutation decreased sharply 5′ upstream and only two substitutions were observed in the 5′ noncoding region of λ1 chain genes.

It was not known whether unrearranged V genes are a target in B cells in which somatic mutation is highly active. Therefore, we examined the unrearranged Vλ1 genes from C6-8-2, E3-19, E11-14, and 5E2. Since Vλ1 loci are supposed to be the germline configuration for both alleles in the parent cell (Sp2/0-Ag14), we assumed that there were three unrearranged Vλ1 genes in these hybridomas, two from the Sp2/0-Ag14 cell and one from the spleen cell (25). Primers were designed to amplify only unrearranged Vλ1 genes (Fig. 1), which were cloned and sequenced. Six cloned from each PCR were subjected to sequencing and only recurrent mutations were considered somatic mutation. As shown in Fig. 6 *A*, eight clones from C6-8-2 showed the same nucleotide sequence as a germline counterpart, whereas four had two recurrent mutations at positions 270 and 313. Essentially similar patterns of mutation to Fig. 6 *A* were observed in the sequences from E11-14 and 5E2 in addition to Sp2/0-Ag14 (data not shown). Therefore, these mutations (positions 270 and 313) would originate from the Vλ1 gene of Sp2/0-Ag14 cells. In the case of E3-19, the recurrent mutations unique to E3-19 were observed at positions 196, 251, 307, and 411 in two clones from the first PCR in addition to those shared with the other hybridomas (positions 270 and 313). These results suggest that unrearranged Vλ1 genes of Sp2/0-Ag14 and E3-19 were a target for the mutator system as in the case of MOPC315 (12).

Frequencies of somatic mutation at various loci of Ig genes are summarized in Table 1. The average frequency in rearranged VH genes was 4.4%. This value is similar to that obtained for rearranged active Vλ1 (3.3%) or inactive Vλ2 (4.2%), which is ~15-fold higher than unrearranged Vλ1 genes. The frequency in the Jλ1-Cλ1 intron was about 1/3 (1.1%) that of VH or Vλ genes, whereas that for the 5′ non-

Figure 3. Nucleotide sequences of the 5′ noncoding region of active rearranged λ1 chain genes from E3-19, E11-14, C6-8-2, and 5E2.

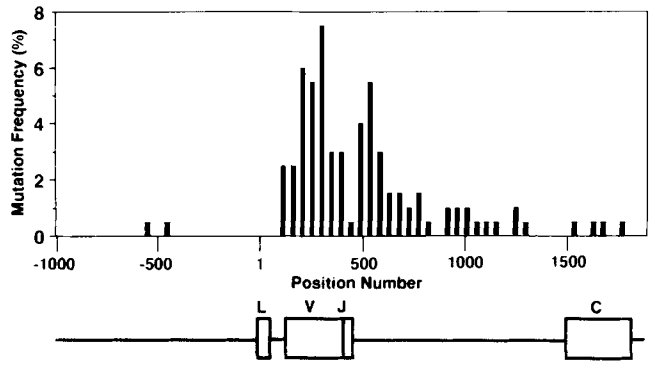


Figure 5. (*Top*) Distribution of somatic mutation in λ1 chain genes. Mutation frequency (%) is the number of mutations per 50 bases sequenced and is plotted against the position number from the start codon ATG. For comparison, reference data (17) are included in this figure. (*Bottom*) Diagram showing rearranged λ1 chain gene.

coding region was 0.04%, only 1/100 that of rearranged VH or Vλ genes. It is also clear that somatic mutation occurs less frequently in 5′ noncoding regions of inactive λ2 chain genes (0.4%) than other loci. However, the frequency of mutation in the 5′ noncoding region of the inactive λ2 chain gene is likely to be higher than that of the active λ1 chain gene although only a limited number of samples was analyzed.

Discussion

The VH promoter and intron enhancer have been shown to be important in induction of somatic mutation using transgenic mice carrying a reporter gene which was driven by these two regions (7). The finding that a non-Ig gene such as the CAT gene became a target of the somatic mutator system suggested that regulation elements such as the promoter and/or enhancer are involved. Since the H chain gene has an intron enhancer and the λ1 chain does not (6), we thought it of interest to determine whether the frequency of somatic mutation of VH and Vλ in the same B cells was different. Although the frequency in Vλ1 seemed to be slightly lower than VH (4.4 compared to 3.3%), the skewed expression of somatic mutation in VH genes, reported by Cumano and Rajewsky (8), was not observed in this study. This discrepancy may have arisen from different immunization conditions since Cumano and Rajewsky used mice that had been injected with an anti-Id mAb, Ac38, before immunization with NP-CGG (8). Therefore, it is unlikely that somatic mutation occurs preferentially in VH genes but not in Vλ1 genes under normal immunization conditions, although an intron enhancer is absent in λ chain genes (26). As shown by our group (7) and by Sohn et al. (27), somatic mutation was observed in transgenes containing only the H chain intron enhancer, although the mutation rate was lower than that of complete H chain genes. Therefore, the intron enhancer may play an essential role in the induction of somatic mutation in H-chain genes, whereas the 3′ enhancer is more important in the case of λ and κ chain genes since λ chain genes which lack an intron enhancer are able to induce mutation with a frequency similar to VH genes (17), and since κ chain transgenes lacking the 3′ enhancer element are not (28).

The lower frequency of somatic mutation in the 5′ noncoding region compared with the Jλ1-Cλ1 intron was clearly evident in our study. The average frequency in the 5′ noncoding region was ~1/30 that of the Jλ1-Cλ1 intron. Since the same region of inactive λ2 chain genes showed a 10-fold higher frequency, although still lower than that of the other

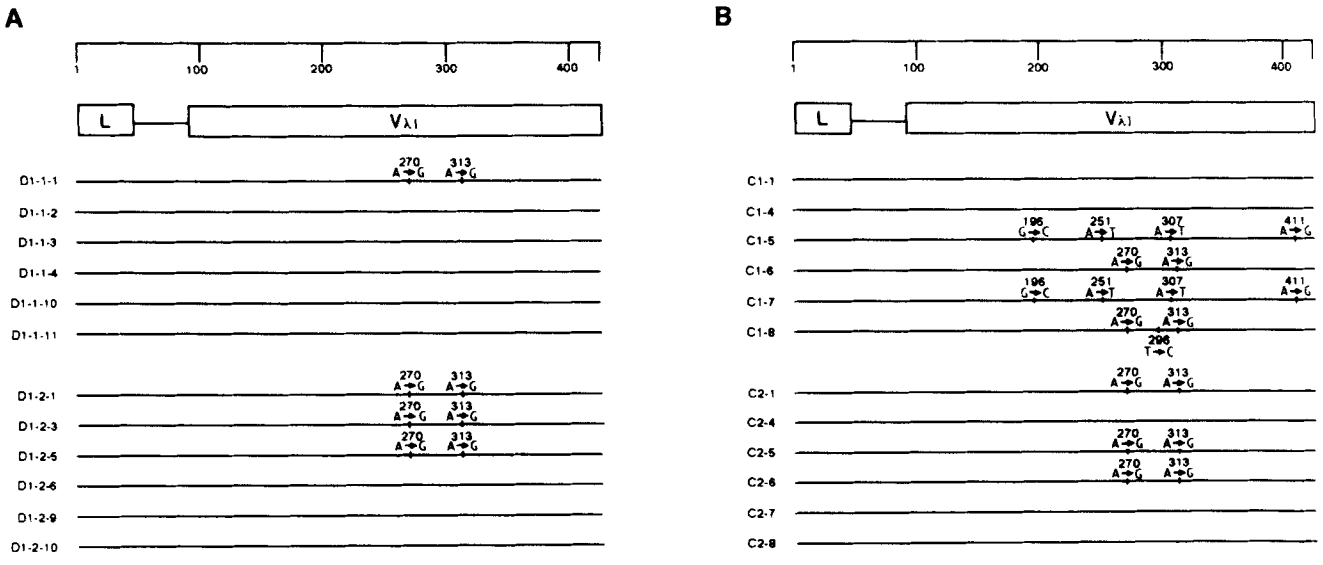


Figure 6. Nucleotide sequences of unrearranged Vλ1 genes from hybridoma, C6-8-2 (*A*) and E3-19 (*B*), producing anti-NP mAbs bearing λ1 chains. Unrearranged Vλ1 DNA was amplified twice independently using Taq DNA polymerase. Six clones from each PCR were subjected to sequencing. Nucleotide sequences identical to their germline counterpart are shown by solid lines and only locations different from the germline are indicated. The position was numbered relative to translation initiation site.

Table 1. *Comparison of Frequency of Somatic Mutation among Ig Gene Loci*

	Rearranged					
	Active			Inactive		Unrearranged
Cells	VH	Vλ1*	5′	Vλ2*	5′	Vλ1
E11-14	23/433	13/441	2/1149	13/420	2/495	0/441
C6-8-2	12/433	9/441	0/1149	-	-	0/441
5E2	13/433	12/441	0/1149	-	-	0/441
E3-19	29/433	24/441	0/1149	22/420	2/495	4/441
Sp2/0	-	-	-	-	-	2/441‡
Average No. of mutations	19.3/433	14.5/441	0.5/1149	17.5/420	2/495	1 /441
Frequency (%)	4.4	3.3	0.04	4.2	0.40	0.23

* Data taken from Motoyama et al. (17).
‡ Not included for calculation of average No. of mutations.

loci, the much lower frequency of active λ1 chain genes can be explained partly in terms of selection of unmutated genes, since we analyzed λ1 chain genes from hybridomas producing λ1 chains. On the other hand, similar low frequencies in the 5′ noncoding regions of the VH gene have been reported by others (10, 11). In the case of H chain genes, mutation frequency decreased immediately 5′ upstream from the cap site. Therefore, it can be generalized that somatic mutation occurs in the 5′ noncoding region with a lower frequency than in other Ig gene loci.

Somatic mutation was not prevented in unrearranged Vλ1 genes from E3-19, and results coincide with the occurrence of somatic mutation in unrearranged Vλ1 and Vλ2 genes of a myeloma, MOPC315 (12). The frequency of mutation in unrearranged Vλ1 in our study was 0.23% which is ~1/15 that of rearranged Vλ1 and of the same order as that of rearranged D-J genes (29). Since both E3-19 and MOPC315 secrete Abs bearing λ chains, it appeared that somatic mutation occurs in unrearranged Vλ genes of B cells producing λ chains. However, we found two nucleotide substitutions in the unrearranged Vλ1 gene in Sp2/0-Ag 14 cells in which λ locus retains the germline configuration (25). Since nine nucleotide substitutions were found in the inactively rearranged κ chain gene (11), the mutator system was expected to be active at a specific developmental stage of Sp2/0-Ag14. Therefore, it can be generalized that somatic mutation occurs in the unrearranged Vλ of B cells where the mutator mechanism is highly activated.

No somatic mutation was found in unrearranged VH and Vκ genes in contrast to Vλ genes (13–16). This may be explained in terms of the different germline structure between the λ locus and the H or κ locus. The λ locus is arranged as Vλ2/Vλx/JCλ2/JCλ4/Eλ2-4/Vλ1/JCλ3/JCλ1/Eλ3-1, where Eλ2-4 and Eλ3-1 are enhancer elements located 15.5 kb downstream of Cλ4 and 35 kb downstream of Cλ1, respectively (6, 30). Since these gene segments are distributed over a rather limited distance (~200 kb) and since two enhancer elements in this locus are active in both κ and λ chain–producing B cells, unrearranged Vλ genes are thought to be present in the open chromatin structure to which the mutator system is accessible. This is not the case for VH and Vκ genes (31, 32).

In a previous paper, we suggested that the signal for induction of somatic mutation resides in the promoter and/or enhancer but not in the V-(D)-J exon, and that targets for the somatic mutator system are genes existing immediately 3′ downstream of the promoter element. The finding that somatic mutation was practically absent in the 5′ noncoding region (0.04%) suggests that the binding of protein factors to the regulatory elements in this region may be essential for induction of somatic mutation. The occurrence of somatic mutation in unrearranged Vλ1 genes, even though of low frequency (0.23%), supports the idea that the 5′ noncoding region is able to induce mutation. Activity of the promoter would be highest when its position relative to the enhancer is optimized by recombination (28). Rogerson et al. (31) proposed a model that predicted the occurrence of mutator factors that bind to the mutation initiation region (MIR) located upstream of promoter. Our previous and present results are consistent with their model, suggesting a pivotal role for this region in the induction of somatic mutation specific to Ig genes.

Other than the 5′ noncoding region, somatic mutation was not detected in CH and Cκ loci (33). In the case of λ1 chain genes, somatic mutation occurred in Vλ1-Jλ1 genes with highest frequency and decreased with distance from Vλ1-

Jλ1 exon into Jλ1-Cλ1 intron, and was observed even in Cλ1. This suggests that a specific signal for prohibiting mutation in the C exon does not reside in the Jλ1-Cλ1 intron or Cλ1 exon. This may be the case for κ and H chain genes and hence the absence of somatic mutation in CH or Cκ genes may be explained by their location some distance from the promoter elements. CH and Cκ exons were separated from V-(D)-J exons by J-C introns of ~6.5 and 3 kb, respectively, which is lengthy in contrast to the short (1.2 kb) Jλ1-Cλ1 intron. Therefore, it is likely that the distribution of somatic mutation 3′ downstream is inversely proportional to the distance from the promoter. Recently, an unusual distribution of somatic mutation was reported in Vκ12.37-Jκ1 gene (34). In this gene, somatic mutation was observed at a high frequency in the 3′ flanking region rather than in the V-J exon. As suggested by the authors, (34) Vκ12.37 gene may lack a *cis*-element or have one which is mutated and determines location where somatic mutation occurs.

In conclusion, somatic mutation can be induced in V genes regardless of whether they are rearranged, although rearranged genes mutate with a higher frequency, more than 15-fold those of unrearranged genes. The signal for induction of somatic mutation may reside in the 5′ noncoding region flanking V genes and the presence of an enhancer at the appropriate location would maximize promoter function. Cooperation of these regulatory elements should induce a high frequency of somatic mutation in V genes, or any genes which are regulated by these elements, in B cells after immunization with T-dependent antigens.

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