# Somatic point mutations in unrearranged immunoglobulin gene segments encoding the variable region of $\lambda$ light chains

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Communicated by H.von Boehmer

Somatic point mutations are usually found in the coding and flanking regions of functionally and aberrantly rearranged immunoglobulin variable region gene segments. Mutations in the unrearranged V gene segments of myelomas or hybridomas have not been described so far. We have cloned and sequenced unrearranged V $\lambda$  gene segments from several cell lines. There were no nucleotide changes in four unrearranged V  $\lambda$  segments: one V  $\lambda 1$  from a  $\lambda 3\text{-producing}$ hybridoma and one V $\lambda$ 2 from a  $\lambda$ 1-producing myeloma (J558) and two V $\lambda$ 2 from a  $\chi$ -producing myeloma (P3X63). However, we found somatic mutations in the unrearranged V $\lambda$  segments from the  $\lambda$ 2-producing myeloma MOPC315. The unrearranged  $V\lambda 1$  gene segment had two mutations in the coding region and the unrearranged  $V\lambda 2$  had one mutation in the 3' flanking region. We also cloned and sequenced the unrearranged J $\lambda$  and C $\lambda$  gene segments of MOPC315 and found no sequence alterations. This is consistent with the notion that the overall mutation rate is not higher in this cell line. Therefore, we suggest that the somatic hypermutation system can use unrearranged V gene segments as substrates. The extensive sequencing required for this work revealed a number of errors in the reported nucleotide sequences of the Ig  $\lambda$  locus in BALB/c mice.

Key words: DNA sequence/ $\lambda$  light chains/MOPC315/somatic mutation/unrearranged immunoglobulin V, J and C gene segments

# Introduction

Immunoglobulin genes are encoded in several segments in the germline -V, J and C in the case of light chains and V, D, J and C in the case of heavy chains. These segments are assembled during B-cell development to form complete transcriptional units. Since more than one equivalent of each particular segment exists, the combinatorial joining of these segments is a major source of the tremendous diversity observed among antibodies (for review see Tonegawa, 1983; Yancopoulos and Alt, 1986).

An additional mechanism which is probably responsible for maturation of antibody quality, is the substitution of single nucleotides (Weigert *et al.*, 1970; Tonegawa, 1976; for review see Tonegawa, 1983 and Manser *et al.*, 1985). A hypermutational system has been postulated to be responsible for these nucleotide substitutions. Mutation frequencies estimated *in vivo* were up to eight orders of magnitude higher than the frequencies estimated for normal mammalian cells (McKean *et al.*, 1984; Sablitzky *et al.*, 1985). Since the degree of selection influencing these estimates is not known, the mutation frequencies represent maximal frequencies. Mutation frequencies observed with one particular cell line *in vitro* were one or two orders of magnitude lower than the *in vivo* estimates (Wabl *et al.*, 1985).

Based on the comparison of rearranged V gene segments with the same V gene segments isolated from non-lymphoid tissue it was suggested that this hypermutational system only acts on or near rearranged V gene segments (Pech *et al.*, 1981). In addtion no mutations have been found in the published sequences of four unrearranged V $\kappa$  segments from plasmacytoma lines in which the rearranged genes had somatic mutations (Nishioka and Leder, 1980; Selsing and Storb, 1981; Gorski *et al.*, 1983).

Here we show that in the plasmacytoma MOPC315 the unrearranged V $\lambda$ 1 and V $\lambda$ 2 gene segments contain two and one base substitutions, respectively. Even though the unrearranged V $\lambda$ segments in several other cell lines do not contain mutations, the finding with MOPC315 suggests that unrearranged V gene segments can be a substrate for the hypermutation system.

# **Results**

We have cloned and sequenced unrearranged V $\lambda$  gene segments derived from the genomic DNA of several cell lines (Figures 1 and 2). The DNA clones and the cell lines are described below.

 $A1-13/eV\lambda 1$  is a DNA clone of an unrearranged V $\lambda 1$  segment from the  $\lambda 3$ -producing hybridoma A1-13. No point mutations have been found in the functional, rearranged V $\lambda 1J\lambda 3$  segment of this line (S.W. and M.Wabl, unpublished). It is not known whether the eV $\lambda 1$  gene segment comes from the spleen cell or the fusion partner, P3X63Ag8.653 (Kearney *et al.*, 1979).

 $Sp7/eV\lambda 2$ -6.8 and  $Sp7/eV\lambda 2$ -7.4 represent DNA clones of unrearranged  $V\lambda 2$  segments from the hybridoma Sp7, which produces  $\lambda 1$  (Köhler and Milstein, 1976). Since the x-producing fusion partner, P3X63Ag8, has a transposon intergrated upstream of the V $\lambda$ 2 segments resulting in two *Eco*RI fragments 6.8 and 7.4 kb in size that are also found in Sp7 (S.W. and B.Johansson, in preparation), these V $\lambda$ 2 gene segments must come from P3X63Ag8. Since P3X63Ag8, in addition to the 6.8- and 7.4-kb fragments, contains a normal-sized V<sub>2</sub> EcoRI fragment,  $V\lambda 2$ -6.8 and  $V\lambda 2$ -7.4 must originate from one  $V\lambda 2$  allele that was duplicated during the process of aneuploidization. It is important to note that these unrearranged gene segments came from a cell line that has been in culture a very long time. The rearranged heavy and light chain genes of P3X63Ag8 have been sequenced, but it is not known whether they contain somatic point mutations in the V regions, because the germline counterparts of these genes have not been sequenced (Hamlyn et al., 1981; Walfield et al., 1981; Bothwell et al., 1981a).

 $J558/eV\lambda 2a$  and  $J558/eV\lambda 2b$  are two independent DNA clones from the plasmacytoma J558 which produces  $\lambda 1$ . Both clones were found identical and are therefore displayed only once as J558/eV $\lambda 2$  (Figure 2). Since J558 ought to contain two unrearranged V $\lambda 2$  segments, these sequences might be derived from both or only one allele. The protein sequences of the expressed heavy and the  $\lambda 1$  light chain give no evidence of mutations in the genes encoding them (Weigert *et al.*, 1970; Schilling *et al.*,

germline  $V\lambda 1$ tctagaatat ggatagtggg tgtttatgac tctggataag cctgaacaat tgatgattaa tgcccctgag ctctgttctt tctagaatat ggatagtggg tgtttatgac tctggataag cctgaacaat tgatgattaa tgcccctgag ctctgttctt  $A1-13/eV\lambda1$  $J558/V\lambda 1J\lambda 3$ totagaatat ggatagtggg tgtttatgac totggataag cotgaacaat tgatgattaa tgococotgag cootgttott M315/eVλ1 tctagaatat ggatagtggg tgtttatgac tctggataag cctgaacaat tgatgattaa tgcccctgag ctctgttctt germline  $V\lambda 1$ agtaacatgt gaacatttac ttgtgtcagt gtagtagatt tcacatgaca tcttataata aacctgtaaa tgaaagtaat A1-13/eVλ1 agtaacatgt gaacatttac ttgtgtcagt gtagtagatt tcacatgaca tcttataata aacctgtaaa tgaaagtaat J558/VA1JA3 agtaacatgt gaacatttac ttgtgtcagt gtagtagatt tcacatgaca tcttataata aacctgtaaa tgaaagtaat M315/eVλ1 agtaacatgt gaacatttac ttgtgtcagt gtagtagatt tcacatgaca tcttataata aacctgtaaa tgaaagtaat 161 germline  $V\lambda 1$ ttgcattact agcccagccc agcccatact aagagttata ttatgtctgt ctcacagcct gctgctgacc aatattgaaa ttgcattact agcccagccc agcccatact aagagttata ttatgtctgt ctcacagcct gctgctgacc aatattgaaa A1-13/eVλ1 ttgcattact ageccagece ageccatact aagagttata ttatgtetgt etcacageet getgetgace aatattgaaa J558/VA1JA3 M315/eVλ1 ttgcattact ageccagece ageccatact aagagttata ttatgtetgt etcacageet getgetgace aatattgaaa 241 agaatagacc tggtttgtga attATGGCCT GGATTTCACT TATACTCTCT CTCCTGGCTC TCAGCTCAGG Tcagcagcct germline  $V\lambda 1$ agaatagacc tggtttgtga attATGGCCT GGATTTCACT TATACTCTCT CTCCTGGCTC TCAGCTCAGG Tcagcagcct A1-13/eVλ1 agaatagacc tggtttgtga attATGGCCT GGATTTCACT TATACTCTCT CTCCTGGCTC TCAGCTCAGG Tcagcagcct J558/V1J13 agaatagacc tggtttgtga attATGGCCT GGATTTCACT TATACTCTCT CTCCTGGCTC TCAGCTCAGG Tcagcagcct M315/eVλ1 321 germline  $V\lambda 1$ ttctacactg cagtgggtat gcaacaatge gcatcttgte tetgatttge tactgatgae tggatttete atetgtttge ttctacactg cagtgggtat gcaacaatgc gcatcttgtc tctgatttgc tactgatgac tggatttctc atctgtttgc A1-13/eVλ1 ttctacactg cagtgggtat gcaacaatgc gcatcttgtc tctgatttgc tactgatgac tggatttctc atctgtttgc J558/Vλ1Jλ3 ttctacactg cagtgggtat gcaacaatgc gcatcttgtc tctgatttgc tactgatgac tggatttctc atctgtttgc M315/eVλ1 germline  $V\lambda 1$ AGGGGCCATT TCCCAGGCTG TTGTGACTCA GGAATCTGCA CTCACCACAT CACCTGGTGA AACAGTCACA CTCACTTGTC A1-13/eVλ1 AGGGGCCATT TCCCAGGCTG TTGTGACTCA GGAATCTGCA CTCACCACAT CACCTGGTGA MACAGTCACA CTCACTTGTC J558/Vλ1Jλ3 AGGGGCCATT TCCCAGGCTG TTGTGACTCA GGAATCTGCA CTCACCACAT CACCTGGTGA AACAGTCACA CTCACTTGTC M315/eVλ1 AGGGGCCCATT TCCCAGGCTG TTGTGACTCA GGAATCTGCA CTCACCACAT CACCTGGTGA AACAGTCACA CTCACTTGTC CTCAAGTAC TGGGGCTGTT ACAACTAGTA ACTATGCCAA CTGGGTCCAA GAAAAACCAG ATCATTTATT CACTGGTCTA germline  $V\lambda 1$ A1-13/eVλ1 GCTCAAGTAC TGGGGCTGTT ACAACTAGTA ACTATGCCAA CTGGGTCCAA GAAAAACCAG ATCATTTATT CACTGGTCTA J558/VA1JA3 GCTCAAGTAC TGGGGCTGTT ACAACTAGTA ACTATGCCAA CTGGGTCCAA GAAAAACCAG ATCATTTATT CACTGGTCTA M315/eVλ1 GCTCAAGTAC TGGGGCTGCT ACAACAAGTA ACTATGCCAA CTGGGTCCAA GAAAAACCAG ATCATTTATT CACTGGTCTA 561 ATAGGTGGTA CCAACAACCG AGCTCCAGGT GTTCCTGCCA GATTCTCAGG CTCCCTGATT GGAGACAAGG CTGCCCTCAC germline  $V\lambda 1$ A1-13/eVλ1 ATAGGTGGTA CCAACAACCG AGCTCCAGGT GTTCCTGCCA GATTCTCAGG CTCCCTGATT GGAGACAAGG CTGCCCTCAC J558/VA1JA3 ATAGGTGGTA CCAACAACCG AGCTCCAGGT GTTCCTGCCA GATTCTCAGG CTCCCTGATT GGAGACAAGG CTGCCCTCAC M315/eVλ1 ATAGGTGGTA CCAACAACCG AGCTCCAGGT GTTCCTGCCA GATTCTCAGG CTCCCTGATT GGAGACAAGG CTGCCCTCAC 641 germline  $V\lambda 1$ CATCACAGGG GCACAGACTG AGGATGAGGC AATATATTTC TGTGCTCTAT GGTACAGCAA CCATTTccac aatgacatgt A1-13/eVλ1 CATCACAGGG GCACAGACTG AGGATGAGGC AATATATTTC TGTGCTCTAT GGTACAGCAA CCATTTCcac aatgacatgt J558/Vλ1Jλ3 CATCACAGGG GCACAGACTG AGGATGAGGC AATATATTTC TGTGCTCTAT GGTACAGCAA CCA M315/eVλ1 CATCACAGGG GCACAGACTG AGGATGAGGC AATATATTTC TGTGCTCTAT GGTACAGCAA CCATTTccac aatgacatgt 721 germline  $V\lambda 1$ gtagatgggg aagtagaaca agaacactct ggtacagtct cataactacc actttcttaa caggtggcta catctcccta A1-13/eVλ1 gtagatgggg aagtagaaca agaacactct ggtacagtct cataactacc atcttcttaa caggtggcta catctcccta M315/eVλ1 gtagatgggg aagtagaaca agaacactct ggtacagtct cataactacc atcttcttaa caggtggcta catctcccta 801 germline  $V\lambda 1$ gtctgttctc ttttactata gagaaattta taaaagctgt tgtctcaatc aataaaaagt tttatttcaa caaattgtat A1-13/eVλ1 gtctgttctc ttttactata gagaaattta taaaagctgt tgtctcaatc aataaaaagt tttatttcaa caaattgtat M315/eVλ1 gtctgttctc ttttactata gagaaattta taaaagctgt tgtctcaatc aataaaaaqt tttatttcaa caaattqtat 881 germline  $V\lambda 1$ aattatgcct tgatgacaag A1-13/eVλ1 aattatgcct tgatgacaag M315/eVλ1 aattatgcct tgatgacaag

Fig. 1. Comparison of the published sequence of germline  $V\lambda 1$  with unrearranged  $V\lambda 1$  gene segments from A1-13 and MOPC315 and  $V\lambda 1$  part of the aberrantly rearranged  $\lambda 3$  gene from J558 ( $V\lambda 1J\lambda 3$  – the J $\lambda 3$  of this clone is shown in Figure 3). Capitial letters indicate the coding regions. Dots indicate disagreements of all our sequences with the published one. The asterisks indicate the position of nucleotide substitutions in the unrearranged  $V\lambda$  segments from MOPC315.

1980). This line also contains an aberrantly rearranged  $\lambda 3$  gene which we have cloned and sequenced and which also contains no somatic mutations (Figures 1 and 3). Thus, no somatic mutations have been demonstrated in J558.

MOPC315/ $eV\lambda 1$  and MOPC315/ $eV\lambda 2$  are DNA clones of unrearranged V $\lambda$  gene segments from the plasmacytoma MOPC315, which produces  $\lambda 2$  and in which both rearranged  $\lambda$ s contain a number of somatic mutations. Six mutations were found in the functional  $\lambda 2$  (Wu *et al.*, 1982; Bothwell *et al.*, 1982) and nine in the aberrant  $\lambda 1$  (Hozumi *et al.*, 1981; Bothwell *et al.*, 1981b). The heavy chain has not yet been analysed.

Figures 1 and 2 show the comparison of the published germline DNA sequence of these gene segments (Tonegawa *et al.*, 1978; Bernard *et al.*, 1978) and the sequence obtained by us. In all these sequences there are a few differences from the published germline sequences (marked with dots in Figures 1 and 2). Because all of our sequences contain these changes, they are most

likely due to errors in the published sequences. However, three differences, two in the coding region of V $\lambda$ 1 from MOPC315 and one in the 3' flanking region of V $\lambda$ 2 from MOPC315 (marked by stars in Figures 1 and 2), cannot be explained by sequencing errors. Each of the two V $\lambda$  sequences from MOPC315 was obtained from two independent phage clones. Thus we believe these nucleotide substitutions are true mutations in unrearranged V $\lambda$  gene segments.

Somatic point mutations in rearranged immunoglobulin genes are usually also found in the J segment and its downstream flanking region. To determine whether the hypermutation system can also use the unrearranged J segments as substrate, we have cloned and sequenced four unrearranged J $\lambda$  segments of MOPC315. Differences were found with the published sequences of J $\lambda$ 2, J $\lambda$ 3 and J $\lambda$ 4 (Blomberg and Tonegawa, 1982; Miller *et al.*, 1982). However, upon comparing our data with unpublished data kindly provided by Dr U.Storb, University of Chicago, we conclude

germline  $V\lambda 2$ gateteacgt gacatettat aataaacetg taaatgaaag taatttgeat taetageeca geceageeca taetaagagt SP7/eVλ2-6.8 SP7/eV2-7.4 gateteacgt gacatettat aataaacetg taaatgaaag taatttgcat tactageeca geecageeca tactaagagt J558/eVλ2 M315/eVλ2 81 germline  $V\lambda 2$ tatattatgt ctgtctcact gcctgctgct gaccaatatt gaaaataata gacttggttt gtgaattATG GCCTGGACTT SP7/eV\2-6.8 tatattatgt ctgtctcact gcctgctgct gaccaatatt gaaaataata gacttggttt gtgaattATG GCCTGGACTT SP7/eV32-7.4 tatattatgt ctgtctcact gcctgctgct gaccaatatt gaaaataata gacttggttt gtgaattATG GCCTGGACTT .T558/eV \2 tatattatgt ctgtctcact gcctgctgct gaccaatatt gaaaataata gacttggttt gtgaattATG GCCTGGACTT M315/eVλ2 tatattatgt ctgtctcact gcctgctgct gaccaatatt gaaaataata gacttggttt gtgaattATG GCCTGGACTT 161 germline  $V\lambda 2$ CACTTATACT CTCTCTCCTG GCTCTCTGCT CAGGtcagca gcctttctac actgcagtgg gtatgcaaca atacacatct SP7/eV2-6.8 CACTTATACT CTCTCTCCTG GCTCTCTGCT CAGGtcagca gcctttctac actgcagtgg gtatgcaaca atacacatct SP7/eV\2-7.4 CACTTATACT CTCTCTCCTG GCTCTCTGCT CAGGtcagca gcctttctac actgcagtgg gtatgcaaca atacacatct J558/eVλ2 CACTTATACT CTCTCTCCTG GCTCTCTGCT CAGGtcagca gcctttctac actgcagtgg gtatgcaaca atacacatct M315/eVλ2 CACTTATACT CTCTCTCCTG GCTCTCTGCT CAGGtcagca gcctttctac actgcagtgg gtatgcaaca atacacatct 241 dermline  $V\lambda 2$ tgtctctgat ttgctactga tgactggatt tcttacctgt ttgcaggAGC CAGTTCCCAG GCTGTTGTGA CTCAGGAATC SP7/eV2-6.8 tgtctctgat ttgctactga tgactggatt tcttacctgt ttgcaggAGC CAGTTCCCAG GCTGTTGTGA CTCAGGAATC SP7/eV2-7.4 tgtctctgat ttgctactga tgactggatt tcttacctgt ttgcaggAGC CAGTTCCCAG GCTGTTGTGA CTCAGGAATC J558/eVλ2 tgtetetgat ttgetactga tgactggatt tettacetgt ttgeaggAGC CAGTTCECAG GETGTTGTGA CTCAGGAATC M315/eVλ2 tgtctctgat ttgctactga tgactggatt tcttacctgt ttgcaggAGC CAGTTCCCAG GCTGTTGTGA CTCAGGAATC germline  $V\lambda 2$ TGCACTCACC ACATCACCTG GTGGAACAGT CATACTCACT TGTCGCTCAA GTACTGGGGC TGTTACAACT AGTAACTATG SP7/eV2-6.8 TGCACTCACC ACATCACCTG GTGGAACAGT CATACTCACT TGTCGCTCAA GTACTGGGGC TGTTACAACT AGTAACTATG SP7/eV2-7.4 TGCACTCACC ACATCACCTG GTGGAACAGT CATACTCACT TGTCGCTCAA GTACTGGGGC TGTTACAACT AGTAACTATG J558/eVλ2 TGCACTCACC ACATCACCTG GTGGAACAGT CATACTCACT TGTCGCTCAA GTACTGGGGC TGTTACAACT AGTAACTATG M315/eVλ2 TGCACTCACC ACATCACCTG GTGGAACAGT CATACTCACT TGTCGCTCAA GTACTGGGGC TGTTACAACT AGTAACTATG germline  $V\lambda 2$ CCAACTGGGT TCAAGAAAAA CCAGATCATT TATTCACTGG TCTAATAGGT GGTACCAGCA ACCGAGCTCC AGGTGTTCCT SP7/eVλ2-6.8 CCAACTGGGT CCAAGAAAAA CCAGATCATT TATTCACTGG TCTAATAGGT GGTACCAGCA ACCGAGCTCC AGGTGTTCCT SP7/eVλ2-7.4 CCAACTGGGT CCAAGAAAAA CCAGATCATT TATTCACTGG TCTAATAGGT GGTACCAGCA ACCGAGCTCC AGGTGTTCCT J558/eVλ2 CCAACTGGGT CCAAGAAAAA CCAGATCATT TATTCACTGG TCTAATAGGT GGTACCAGCA ACCGAGCTCC AGGTGTTCCT  $M315/eV\lambda2$ CCAACTGGGT CCAAGAAAAA CCAGATCATT TATTCACTGG TCTAATAGGT GGTACCAGCA ACCGAGCTCC AGGTGTTCCT germline  $V\lambda 2$ STCAGATTCT CAGGCTCCCT GATTGGAGAC AAGGCTGCCC TCACCATCAC AGGGGCACAG ACTGAGGATG ATGCAATGTA SP7/eVλ2-6.8 STCAGATTCT CAGGCTCCCT GATTGGAGAC AAGGCTGCCC TCACCATCAC AGGGGCACAG ACTGAGGATG ATGCAATGTA SP7/eVλ2-7.4 STCAGATTCT CAGGCTCCCT GATTGGAGAC AAGGCTGCCC TCACCATCAC AGGGGCACAG ACTGAGGATG ATGCAATGTA J558/eVλ2 STCAGATTCT CAGGCTCCCT GATTGGAGAC AAGGCTGCCC TCACCATCAC AGGGGCACAG ACTGAGGATG ATGCAATGTA M315/eVλ2 STCAGATTCT CAGGETCECT GATTGGAGAC AAGGETGECE TCACCATCAC AGGGGCACAG ACTGAGGATG ATGCAATGTA 561 germline  $V\lambda 2$ TTTCTGTGCT CTATGGTACA GCACCCATTT ccacaatgac atgtgtagat ggggaagtag aacaagaaca ctctggtaca SP7/eV2-6.8 TTTCTGTGCT CTATGGTACA GCACCCATTT ccacaatgac atgtgtagat ggggaagtag aacaagaaca ctctggtaca SP7/eVλ2-7.4 TTTCTGTGCT CTATGGTACA GCACCCATTT ccacaatgac atgtgtagat ggggaagtag aacaagaaca ctctggtaca J558/eVλ2 TTTCTGTGCT CTATGGTACA GCACCCATTT ccacaatgac atgtgtagat ggggaagtag aacaagaaca ctctggtaca M315/eVλ2 TTTCTGTGCT CTATGGTACA GCACCCATTT ccacaatgac atgtgtagat ggggaagtag aacaagaaca ctctggtaca 641 germline  $V\lambda 2$ gtctca c taccatette ttaacaggtg getacatgte eetagtetgt tetetttae tatagagaaa tttataaaag SP7/eVλ2-6.8 gtotoataac taccatotto ttaacaggtg gotacatgto cotagtotgt tototttac tatagagaaa tttataaaag SP7/eVλ2-7.4 gtctcataac taccatcttc ttaacaggtg gctacatgtc cctagtctgt tctcttttac tatagagaaa tttataaaag J558/eVλ2 gtctcataac taccatcttc ttaacaggtg gctacatgtc cctagtctgt tctcttttac tatagagaaa tttataaaag gtetcataac taccatette ttaacaggtg getacatgte ectagtetgt tetetattae tatagagaaa tttataaaag M315/eVλ2 721 germline  $V\lambda 2$ ctgttgtctc gagcaacaaa aagtttta t tcaacaaatt gtataataat tatgccttga tgacaagct SP7/eV\2-6.8 ctgttgtctc gagcaacaaa aagttttatt tcaacaaatt gtataataat tatgccttga tgacaagct SP7/eV2-7.4 ctgttgtctc gagcaacaaa aagttttatt tcaacaaatt gtataataat tatgccttga tgacaagct J558/eVλ2 ctgttgtctc gagcaacaaa aagttttatt tcaacaaatt gtataataat tatgccttga tgacaagct M315/eVλ2 ctgttgtctc gagcaacaaa aagttttatt tcaacaaatt gtataataat tatgccttga tgacaagct

Fig. 2. Comparison of the published sequence of germline  $V\lambda^2$  with unrearranged  $V\lambda^2$  gene segments from Sp7 (derived from the fusion partner P3X63Ag8 – see text), J558 and MOPC315. Symbols are used as in Figure 1. The difference at position 411 does not change the assumed germline amino acid sequence.

that the differences were due to errors in the published sequences. Figure 3 displays the sequences of the J $\lambda$ s from MOPC315 and the unpublished germline sequences of unrearranged J $\lambda$  segments.

Can the mutations in the unrearranged V $\lambda$  segments be explained by a generally higher mutation rate of MOPC315? To address this question we sequenced four nonrearranged C $\lambda$  gene segments. As shown in Figure 4, no differences between the sequences of MOPC315 and the published germline sequences were found for C $\lambda$ 1, C $\lambda$ 2 and C $\lambda$ 3 (Selsing *et al.*, 1982). Our sequence of C $\lambda$ 4 from MOPC315 contained some differences from the published germline sequence. But cloning and sequencing of C $\lambda$ 4 from J558, showed it to be identical to C $\lambda$ 4 from MOPC315. Thus, we conclude, that the four J $\lambda$  and C $\lambda$  gene segments of MOPC315 contain no somatic mutations.

### Discussion

The existence of a mechanism which introduces point mutations at high frequency into the coding and flanking regions of rearranged immunoglobulin VJ or VDJ gene segments is now generally accepted [for review see Tonegawa (1983) and Manser *et al.* (1985)]. Based on the limited sequencing data on murine unrearranged Vx gene segments from plasmacytomas, it has been suggested that only rearranged V segments provide a substrate for the hypermutation system (Nishioka and Leder, 1980; Selsing and Storb, 1981; Gorski *et al.*, 1983). It appears, however, that this is not a general rule. Although we did not find somatic mutations in four unrearranged V $\lambda$  segments, a total of three nucleotide substitutions were found in the two unrearranged V $\lambda$ segments from MOPC315. 1

germline Jλ1	ggatcctggg	aagaaggatc	tttcagtgat	gtcaccacct	tccaagaatt	accaggagct	gcatacatca	cagatgcaac
M315/eJλ1	ggatcctggg 81	aagaaggatc	tttcagtgat	gtcaccacct	tccaagaatt	accaggagct	gcatacatca	cagatgcaac
germline Jλ1	ttgagaataa	aatgcatgca	aggttttttg	catgagtcta	tatcacagtg	CTGGGTGTTC	GGTGGAGGAA	CCAAACTGAC
M315/eJλ1	ttgagaataa 161	aatgcatgca	aggttttttg	catgagtcta	tatcacagtg	CTGGGTGTTC	GGTGGAGGAA	CCAAACTGAC
germline Jλ1	TGTCCTAGGT	gagtgactcc	ttcctccttt	gttattgttc	tctccaagac	ttgaggtgct	ttttgttgta	tactttccct
M315/eJλ1	TGTCCTAGGT 241	gagtgactcc	ttcctccttt	gttattgttc	tctccaagac	ttgaggtgct	ttttgttgta	tactttccct
germline Jλ1	ttctgtattc	tgcttcatac	ctatacttca	cactaggtaa	agaatttctt	tcttctctag	a	
M315/eJλ1	ttctgtattc	tgcttcatac	ctatacttca	cactaggtaa	agaatttctt	tcttctctag	a	
cermline I)?	1	taattataaa	atazaatazt	2			<b>.</b>	
M215/or)2	taccacccac	tgetteteaa	glyayylcal	ageteeacee	attgtageta	getagtagtt	tgat caget	cagetgtgag
M315/e5 x2	81	tgetteteaa	gtgaggtCat	agetecacee	attgtagcta	gctagtagtt	tgattcagct	cagctgtgag
germline JA2	agaacaggac	caggtgctgg	ccccataggt	tttgggttgg	gttttagtca	ttgtgtTATG	TTTTCGGCGG	TGGAACCAAG
M315/eJ X2	agaacaggac	caggtgctgg	ccccataggt	tttgggttgg	gttttagtca	ttgtgtTATG	TTTTCGGCGG	TGGAACCAAG
cormline T)2		MACCMaageta	****	**				
M315/01)2	GTCACIGICC	TAGGILLAGULA	gttttaaagt	LL ++				
	1							
germline Jλ3	cacccacttc	aagtgaggtc	acagctccac	ccattgtagc	tagctagtag	tttgattcag	tgcagctgtg	agagaacagg
M315/eJλ3	cacccacttc 81	aagtgaggtc	acagetecae	ccattgtagc	tagctagtag	tttgattcag	tgcagctgtg	agagaacagg
germline Jλ3	cccaggtgct	tgccccacag	gtttagggtt	gggtttcagt	cactgtgg <b>TT</b>	TATTTTCGGC	AGTGGAACCA	AGGTCACTGT
M315/eJλ3 J558/Vλ1Jλ3	cccaggtgct	tgccccacag	gtttagggtt	gggtttcagt	cactgtgg <b>TT</b> TT	TATTTTCGGC TATTTTCGGC	AGTGGAACCA AGTGGAACCA	AGGTCACTGT AGGTCACTGT
	161							
germiine JA3	CCTAGGTaag	tggctttaat	gcttcttcct	aataagteta	ggccttgtta	tcttgcaagg	gtcatttatc	tctctctgga
M315/EUA3 T550/W11T12	CCTAGGTaag	tggctttaat	gettetteet	aataagteea	ggccttgtta	tcttgcaagg	gtcatttatc	tetet gga
5557 4 10 73	CCIAGGIAAG	Lygerlaat	gettetteet	aataagteea	ggeettgtta	tettgeaagg	gccatttatc	tetet gga
	1							
yermine JA4	yaatattaca	gtgatgtcac	cacca ccta	ggatcacccc	cacctacaca	cacagatgca	actggaaaat	aaggtacatg
M315/eJ A4	81	gtgatgtcac	caccatccta	ggatcacccc	cacctacaca	cacagatgca	actggaaaat	aaggtacatg
germiine J∧4	cagagttttt	tgcattagac	tatatcagtg	TTGGGTGTTC	GGAGGTGGAA	CCAGATTGAC	TGTCCTAGAT	gagtgactcc
M315/eJA4	cagagttttt	tgcattagac	tatatcagtg	<b>tTGGGTGTTC</b>	GGAGGTGGAA	CCAGATTGAC	TGTCCTAGAT	gagtgactcc
germline $J\lambda 4$	161 tccctccttt	gttattattg	tctccaagct	t				
m313/eJ A4	LCCCLCCLLL	greattattg	tetecaaget	τ				
		5 5	<b>,</b>	-				

Fig. 3. Comparison of the published sequences of germlin  $J\lambda 1 - J\lambda 4$  with the unrearranged  $J\lambda 1 - J\lambda 4$  from MOPC315 and the J\lambda3 part of the aberrantly rearranged  $\lambda 3$  gene from J558 (V $\lambda 1J\lambda 3$  — see Figure 1). The J558  $\lambda 3$  gene is nonfunctional due to a single nucleotide deletion at the VJ junction. The dots indicate errors in the published sequence (see text).

The plasmacytoma MOPC315 was induced in a mouse originating from the seventh backcross of C57BL/6 onto the BALB/c background (Sirishina and Eisen, 1971). Since the unrearranged V $\lambda$  segments in MOPC315 are located on different chromosomal homologues (Hozumi *et al.*, 1982), it is possible that either V $\lambda$ 1 or V $\lambda$ 2 is derived from C57BL/6. The coding region of V $\lambda$ 1 in both strains is identical (Bothwell *et al.*, 1982). Since the 3' flanking region of V $\lambda$ 2 from C57BL/6 has not been sequenced the two strains might be different in that region. The chance that the 3' flanking region of V $\lambda$ 2 is derived from C57BL/6 has not been sequenced the two strains might be different in that region. The chance that the 3' flanking region of V $\lambda$ 2 is derived from C57BL/6 is, however, only 1/128. Thus, at least two, and most likely all three, of the identified differences are somatic mutations.

In rearranged V region gene segments, somatic mutations extend through the J segment into the flanking region downstream of J (Manser *et al.*, 1985). Therefore one might expect to find substitutions in the unrearranged J $\lambda$  segments of MOPC315. None were found in the four J $\lambda$  segments we sequenced. We also found

no mutations in four different unrearranged  $C\lambda$  gene segments from MOPC315. The sum of the nucleotides sequenced in these cases is more that 2700 (1000 for J, 1700 for the C segments). If the changes in the unrearranged V segments were due to a generally elevated mutation rate in MOPC315 we would have expected also to find some nucleotide changes in the unrearranged J and C segments. We cannot entirely exclude the possibility that the changes we have found are due to spontaneous mutations. However, because of the fact that these nucleotide substitutions occur in or close to variable region gene segments - DNA segments which are known to be targets for a hypermutational systems under certain conditions - we believe that the nucleotide substitutions we have found in the unrearranged V $\lambda$  gene segments of MOPC315 were introduced by the hypermutation system. The reason why somatic mutations of unrearranged V gene segments are only found in MOPC315 up to now could be that this plasmacytoma, which has an extremely high number

germline Cλ1 M315/eCλ1	l ttgaccttct ttgaccttct	cttacttcat cttacttcat	cctgca <b>GGCC</b> cctgca <b>GGCC</b>	AGCCCAAGTC AGCCCAAGTC	TTCGCCATCA TTCGCCATCA	GTCACCCTGT GTCACCCTGT	TTCCACCTTC TTCCACCTTC	CTCTGAAGAG CTCTGAAGAG
germline Cλ1 M315/eCλ1	81 CTCGAGACTA CTCGAGACTA	ACAAGGCCAC ACAAGGCCAC	ACTGGTGTGT ACTGGTGTGT	ACGATCACTG ACGATCACTG	ATTTCTACCC ATTTCTACCC	AGGTGTGGTG AGGTGTGGTG	ACAGTGGACT ACAGTGGACT	GGAAGGTAGA GGAAGGTAGA
germline Cλ1 M315/eCλ1	TGGTACCCCT TGGTACCCCT	GTCACTCAGG GTCACTCAGG	GTATGGAGAC GTATGGAGAC	AACCCAGCCT AACCCAGCCT	TCCAAACAGA TCCAAACAGA	GCAACAACAA GCAACAACAA	GTACATGGCT GTACATGGCT	AGCAGCTACC AGCAGCTACC
germline Cλ1 M315/eCλ1	TGACCCTGAC TGACCCTGAC 321	AGCAAGAGCA AGCAAGAGCA	TGGGAAAGGC TGGGAAAGGC	ATAGCAGTTA ATAGCAGTTA	CAGCTGCCAG CAGCTGCCAG	GTCACTCATG GTCACTCATG	AAGGTCACAC AAGGTCACAC	TGTGGAGAAG TGTGGAGAAG
germline Cλ1 M315/eCλ1	AGTTTGTCCC AGTTTGTCCC	GTGCTGACTG GTGCTGACTG	<b>TTCCTAG</b> gtc <b>TTCCTAG</b> gtc	atctaacctt atctaacctt	cattttaccc cattttaccc	acagagg acagagg		
germline Cλ2 M315/eCλ2	l accaatccct accaatccct	tcttttattc tcttttattc	gcaca <b>GGTCA</b> gcaca <b>GGTCA</b>	GCCCAAGTCC GCCCAAGTCC	ACTCCCACTC ACTCCCACTC	TCACCGTGTT TCACCGTGTT	TCCACCTTCC TCCACCTTCC	TCTGAGGAGC TCTGAGGAGC
germline Cλ2 M315/eCλ2	TCAAGGAAAA TCAAGGAAAA	САААGССАСА САААGССАСА	CTGGTGTGTC CTGGTGTGTC	TGATTTCCAA TGATTTCCAA	CTTTTCCCCG CTTTTCCCCG	AGTGGTGTGA AGTGGTGTGA	CAGTGGCCTG CAGTGGCCTG	GAAGGCAAAT GAAGGCAAAT
germline Cλ2 M315/eCλ2	GGTACACCTA GGTACACCTA	TCACCCAGGG TCACCCAGGG	TGTGGACACT TGTGGACACT	TCAAATCCCA TCAAATCCCA	CCAAAGAGGG CCAAAGAGGGG	CAACAAGTTC CAACAAGTTC	ATGGCCAGCA ATGGCCAGCA	GCTTCCTACA GCTTCCTACA
germline Cλ2 M315/eCλ2	241 TTIGACATCG TTIGACATCG	GACCAGTGGA GACCAGTGGA	<b>датстсасаа</b> <b>датстсасаа</b>	CAGTTTTACC CAGTTTTACC	tgtcaagtta Tgtcaagtta	CACATGAAGG CACATGAAGG	GGACACTGTG GGACACTGTG	GAGAAGAGTC GAGAAGAGTC
germline Cλ2 M315/eCλ2	321 TGTCTCCTGC TGTCTCCTGC	AGAATGTCTC AGAATGTCTC	TAAgaaccca TAAgaaccca	ggtttctcct ggtttctcct	tagcctggg a tagcctggg a	aacc		
germline Cλ3 M315/eCλ3	l atcaatccct atcaatccct	tctttcattc tctttcattc	acaca <b>GGTCA</b> acaca <b>GGTCA</b>	GCCCAAGTCC GCCCAAGTCC	ACTCCCACAC ACTCCCACAC	TCACCATGTT TCACCATGTT	TCCACCTTCC TCCACCTTCC	CCTGAGGAGC CCTGAGGAGC
germline Cλ3 M315/eCλ3	81 TCCAGGAAAA TCCAGGAAAA	CAAAGCCACA CAAAGCCACA	CTCGTGTGTGTC CTCGTGTGTGTC	TGATTTCCAA TGATTTCCAA	TTTTTCCCCA TTTTTCCCCA	AGTGGTGTGA AGTGGTGTGA	CAGTGGCCTG CAGTGGCCTG	GAAGGCAAAT GAAGGCAAAT
germline Cλ3 M315/eCλ3	161 GGTACACCTA GGTACACCTA	TCACCCAGGG TCACCCAGGG	TGTGGACACT TGTGGACACT	TCAAATCCCA TCAAATCCCA	CCAAAGAGGA CCAAAGAGGA	CAACAAGTAC CAACAAGTAC	ATGGCCAGCA ATGGCCAGCA	GCTTCTTACA GCTTCTTACA
germline Cλ3 M315/eCλ3	241 TTTGACATCG TTTGACATCG	GACCAGTGGA GACCAGTGGA	<b>GATCTCACAA</b> GATCTCACAA	CAGTTTTACC CAGTTTTACC	TGCCAAGTTA TGCCAAGTTA	CACATGAAGG CACATGAAGG	GGACACTGTG GGACACTGTG	GAGAAGAGTC GAGAAGAGTC
germline Cλ3 M315/eCλ3	321 TGTCTCCTGC TGTCTCCTGC	AGAATGTCTC AGAATGTCTC	<b>ТАА</b> дадссса <b>ТАА</b> дадссса	ggtttctcct ggtttctcct	tagcctagg a tagcctagg a	acc acc		
germline Cλ4 J558/eCλ4 M315/eCλ4	l ggatccctac	tgaaagacca tgaaagacca	tgac agattctgac agattctgac	cttctctttt cttctctttt	tccatcttgc tccatcttgc tccatcttgc	aGGCCAACCC aGGCCAACCC aGGCCAACCC	AAGGCTACAC AAGGCTACAC AAGGCTACAC	CCTCAGTTAA CCTCAGTTAA CCTCAGTTAA
germline Cλ4 J558/eCλ4 M315/eCλ4	81 TCTGTTCCCA TCTGTTCCCA	CCTTCCTCTG CCTTCCTCTG CCTTCCTCTG	AAGAGCTCAA AAGAGCTCAA AAGAGCTCAA	<b>GACTAAAAAG</b> <b>GACTAAAAAG</b> GACTAAAAAG	GCCACACTGG GCCACACTGG GCCACACTGG	TGTGTATGAT TGTGTATGAT TGTGTATGAT	CACTGAGTTC CACTGAGTTC CACTGAGTTC	TACGCAGCTG TACGCAGCTG TACGCAGCTG
germline C\4 J558/eC\4 M315/eC\4	161 CTGTGAGAGT CTGTGAGAGT	GGCCTGGAAG G CCTGGAAG G CCTGGAAG	GCAGATGGTA GCAGATGGTA CCAGATGGTA	CCCCTTTCAC CCCCTTTCAC	TCAGGGTGTA TCAGGGTGTA TCAGGGTGTA	GAGACTACCC GAGACTACCC CAGACTACCC	AGCCTCCCAA AGCCTCCCAA	ACAGAGGGAC ACAGAGGGAC
germline C\4 J558/eC\4 M315/eC\4	241 AACATGGCTA AACATGGCTA AACATGGCTA	GCAGTTACCT GCAGTTACCT GCAGTTACCT	GCTCTTCACA GCTCTTCACA GCTCTTCACA	GCAGAAGCGT GCAGAAGCGT GCAGAAGCGT	GGGAATCTCA GGGAATCTCA GGGAATCTCA	TAGCAGTTAC TAGCAGTTAC TAGCAGTTAC	AGCTGCCATG AGCTGCCATG AGCTGCCATG	TCACTCATGA TCACTCATGA TCACTCATGA
germline Cλ4 J558/eCλ4	321 AGGGCAACA AGGG AACAC	TGTGGAGAAG	AGTTTGTCCC AGTTTGTCCC	GTGCTGAGTG GTGCTGAGTG GTGCTCAGTG	TTCCTAGgtc TTCCTAGgtc	atctgaccct	caccttaccc	acagagg acagaggctg
m315/eCλ4 J558/eCλ4 M315/eCλ4	401 agatcagaaa agatcagaaa	catgccaaag catgccaaag	tatgccttta tatgccttta	gtattttt gtattttt	ITUUTAGGEC	accugaccet	CACCUTACCC	αταγαγγστα

Fig. 4. Comparison of published sequences of germline  $C\lambda 1 - C\lambda 4$  with unrearranged  $C\lambda 1 - C\lambda 4$  from MOPC315 and  $C\lambda 4$  from J558. The dots indicate differences in our sequences from the published ones.

of somatic mutations in the rearranged  $\lambda$  genes, has been longer in a mutable state before it became a tumour or that it was different from other B cells in the quantity of the hypermutational system. In both cases the mutational mechanism might have leaked through onto a normally weak substrate.

It has been suggested that the hypermutation system recognizes a sequence within the JC intron and acts at a certain distance upstream from it. This was based on the findings that oncogenes juxtaposed to the heavy chain switch region due to a translocation contained multiple nucleotide substitutions (Rabbits *et al.*, 1983; Showe *et al.*, 1985; Carè *et al.*, 1986). On the basis of our results, we argue that the recognition site is not close to the JC intron, since the V $\lambda$ s are certainly separated by > 12 kb from the C $\lambda$  clusters (Selsing *et al.*, 1982) while the J $\lambda$ C $\lambda$  introns are only ~ 1.2 kb long. It is more reasonable to assume that recognition sites are in or near the V $\lambda$  segments themselves.

#### Materials and methods

## Cell lines

A 1-13 and Sp7 (Köhler and Milstein, 1976) were grown in IMDM medium supplemented with fetal bovine serum. J558 and MOPC315 were grown as subcutaneous tumours.

#### Molecular cloning and sequencing

Isolation of high mol. wt DNA, cloning into  $\lambda$ gtwes· $\lambda$ B, subcloning of appropriate restriction fragments into M13mp18 and M13mp19 and sequencing by the dideoxy method was done according to published procedures (Smith, 1980; Maniatis *et al.*, 1982; Yannish-Perron *et al.*, 1985). The probes used for cloning and subcloning have been described (Bothwell *et al.*, 1981b; Weiss *et al.*, 1985).

# Acknowledgements

We would like to thank B.Johansson and B.Grossenbacher for expert technical assistance, C.Brügger for composing the Figures, J.Hossmann and C.Plattner for secretarial assistance and Drs C.Steinberg and M.Cohn for critical reading of the manuscript. The Basel Institute for Immunology was founded and is supported by F.Hoffmann-La Roche, Ltd, Basel, Switzerland.

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Received on December 19, 1986; revised on February 5, 1987