The EMBO Journal vol.6 no.4 pp.927–932, 1987

Somatic point mutations in unrearranged immunoglobulin gene segments encoding the variable region of λ light chains

Siegfried Weiss and Gillian E.Wu

Basel Institute for Immunology, Grenzacherstrasse 487, CH 4005 Basel, Switzerland

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 Vλ gene segments from several cell lines. There were no nucleotide changes in four unrearranged Vλ segments: one Vλ1 from a λ3-producing hybridoma and one Vλ2 from a λ1-producing myeloma (J558) and two Vλ2 from a κ-producing myeloma (P3X63). However, we found somatic mutations in the unrearranged Vλ segments from the λ2-producing myeloma MOPC315. The unrearranged Vλ1 gene segment had two mutations in the coding region and the unrearranged Vλ2 had one mutation in the 3′ flanking region. We also cloned and sequenced the unrearranged Jλ and Cλ 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 λ locus in BALB/c mice.

Key words: DNA sequence/λ 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 addition no mutations have been found in the published sequences of four unrearranged Vκ 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 Vλ1 and Vλ2 gene segments contain two and one base substitutions, respectively. Even though the unrearranged Vλ 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 Vλ 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*λ1 is a DNA clone of an unrearranged Vλ1 segment from the λ3-producing hybridoma A1-13. No point mutations have been found in the functional, rearranged Vλ1Jλ3 segment of this line (S.W. and M.Wabl, unpublished). It is not known whether the eVλ1 gene segment comes from the spleen cell or the fusion partner, P3X63Ag8.653 (Kearney *et al.*, 1979).

*Sp7/eV*λ*2-6.8* and *Sp7/eV*λ*2-7.4* represent DNA clones of unrearranged Vλ2 segments from the hybridoma Sp7, which produces λ1 (Köhler and Milstein, 1976). Since the κ-producing fusion partner, P3X63Ag8, has a transposon intergrated upstream of the Vλ2 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 Vλ2 gene segments must come from P3X63Ag8. Since P3X63Ag8, in addition to the 6.8- and 7.4-kb fragments, contains a normal-sized Vλ2 *Eco*RI fragment, Vλ2-6.8 and Vλ2-7.4 must originate from one Vλ2 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*λ*2a* and *J558/eV*λ*2b* are two independent DNA clones from the plasmacytoma J558 which produces λ1. Both clones were found identical and are therefore displayed only once as J558/eVλ2 (Figure 2). Since J558 ought to contain two unrearranged Vλ2 segments, these sequences might be derived from both or only one allele. The protein sequences of the expressed heavy and the λ1 light chain give no evidence of mutations in the genes encoding them (Weigert *et al.*, 1970; Schilling *et al.*,

© IRL Press Limited, Oxford, England

```
                    1
germline Vλ1        tctagaatat ggatagtggg tgtttatgac tctggataag cctgaacaat tgatgattaa tgcccctgag ctctgttctt
A1-13/eVλ1          tctagaatat ggatagtggg tgtttatgac tctggataag cctgaacaat tgatgattaa tgcccctgag ctctgttctt
J558/Vλ1Jλ3         tctagaatat ggatagtggg tgtttatgac tctggataag cctgaacaat tgatgattaa tgcccctgag ctctgttctt
M315/eVλ1           tctagaatat ggatagtggg tgtttatgac tctggataag cctgaacaat tgatgattaa tgcccctgag ctctgttctt
                   81
germline Vλ1        agtaacatgt gaacatttac ttgtgtcagt gtagtagatt tcacatgaca tcttataata aacctgtaaa tgaaagtaat
A1-13/eVλ1          agtaacatgt gaacatttac ttgtgtcagt gtagtagatt tcacatgaca tcttataata aacctgtaaa tgaaagtaat
J558/Vλ1Jλ3         agtaacatgt gaacatttac ttgtgtcagt gtagtagatt tcacatgaca tcttataata aacctgtaaa tgaaagtaat
M315/eVλ1           agtaacatgt gaacatttac ttgtgtcagt gtagtagatt tcacatgaca tcttataata aacctgtaaa tgaaagtaat
                  161
germline Vλ1        ttgcattact agcccagccc agcccatact aagagttata ttatgtctgt ctcacagcct gctgctgacc aatattgaaa
A1-13/eVλ1          ttgcattact agcccagccc agcccatact aagagttata ttatgtctgt ctcacagcct gctgctgacc aatattgaaa
J558/Vλ1Jλ3         ttgcattact agcccagccc agcccatact aagagttata ttatgtctgt ctcacagcct gctgctgacc aatattgaaa
M315/eVλ1           ttgcattact agcccagccc agcccatact aagagttata ttatgtctgt ctcacagcct gctgctgacc aatattgaaa
                  241
germline Vλ1        agaatagacc tggtttgtga attATGGCCT GGATTTCACT TATACTCTCT CTCCTGGCTC TCAGCTCAGG Tcagcagcct
A1-13/eVλ1          agaatagacc tggtttgtga attATGGCCT GGATTTCACT TATACTCTCT CTCCTGGCTC TCAGCTCAGG Tcagcagcct
J558/Vλ1Jλ3         agaatagacc tggtttgtga attATGGCCT GGATTTCACT TATACTCTCT CTCCTGGCTC TCAGCTCAGG Tcagcagcct
M315/eVλ1           agaatagacc tggtttgtga attATGGCCT GGATTTCACT TATACTCTCT CTCCTGGCTC TCAGCTCAGG Tcagcagcct
                  321
germline Vλ1        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           ttctacactg cagtgggtat gcaacaatgc gcatcttgtc tctgatttgc tactgatgac tggatttctc atctgtttgc
                  401
germline Vλ1        aGGGGCCATT TCCCAGGCTG TTGTGACTCA GGAATCTGCA CTCACCACAT CACCTGGTGA AACAGTCACA CTCACTTGTC
A1-13/eVλ1          aGGGGCCATT TCCCAGGCTG TTGTGACTCA GGAATCTGCA CTCACCACAT CACCTGGTGA AACAGTCACA CTCACTTGTC
J558/Vλ1Jλ3         aGGGGCCATT TCCCAGGCTG TTGTGACTCA GGAATCTGCA CTCACCACAT CACCTGGTGA AACAGTCACA CTCACTTGTC
M315/eVλ1           aGGGGCCATT TCCCAGGCTG TTGTGACTCA GGAATCTGCA CTCACCACAT CACCTGGTGA AACAGTCACA CTCACTTGTC
                  481
germline Vλ1        GCTCAAGTAC TGGGGCTGTT ACAACTAGTA ACTATGCCAA CTGGGTCCAA GAAAAACCAG ATCATTTATT CACTGGTCTA
A1-13/eVλ1          GCTCAAGTAC TGGGGCTGTT ACAACTAGTA ACTATGCCAA CTGGGTCCAA GAAAAACCAG ATCATTTATT CACTGGTCTA
J558/Vλ1Jλ3         GCTCAAGTAC TGGGGCTGTT ACAACTAGTA ACTATGCCAA CTGGGTCCAA GAAAAACCAG ATCATTTATT CACTGGTCTA
M315/eVλ1           GCTCAAGTAC TGGGGCTGCT ACAACAAGTA ACTATGCCAA CTGGGTCCAA GAAAAACCAG ATCATTTATT CACTGGTCTA
                            *           *
                  561
germline Vλ1        ATAGGTGGTA CCAACAACCG AGCTCCAGGT GTTCCTGCCA GATTCTCAGG CTCCCTGATT GGAGACAAGG CTGCCCTCAC
A1-13/eVλ1          ATAGGTGGTA CCAACAACCG AGCTCCAGGT GTTCCTGCCA GATTCTCAGG CTCCCTGATT GGAGACAAGG CTGCCCTCAC
J558/Vλ1Jλ3         ATAGGTGGTA CCAACAACCG AGCTCCAGGT GTTCCTGCCA GATTCTCAGG CTCCCTGATT GGAGACAAGG CTGCCCTCAC
M315/eVλ1           ATAGGTGGTA CCAACAACCG AGCTCCAGGT GTTCCTGCCA GATTCTCAGG CTCCCTGATT GGAGACAAGG CTGCCCTCAC
                  641
germline Vλ1        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 Vλ1        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 Vλ1        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 aataaaaagt tttatttcaa caaattgtat
                  881
germline Vλ1        aattatgcct tgatgacaag
A1-13/eVλ1          aattatgcct tgatgacaag
M315/eVλ1           aattatgcct tgatgacaag
```

Fig. 1. Comparison of the published sequence of germline Vλ1 with unrearranged Vλ1 gene segments from A1−13 and MOPC315 and Vλ1 part of the aberrantly rearranged λ3 gene from J558 (Vλ1Jλ3 − the Jλ3 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 Vλ segments from MOPC315.

1980). This line also contains an aberrantly rearranged λ3 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/eVλ1 and *MOPC315/eVλ2* are DNA clones of unrearranged Vλ gene segments from the plasmacytoma MOPC315, which produces λ2 and in which both rearranged λs contain a number of somatic mutations. Six mutations were found in the functional λ2 (Wu *et al.*, 1982; Bothwell *et al.*, 1982) and nine in the aberrant λ1 (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 Vλ1 from MOPC315 and one in the 3′ flanking region of Vλ2 from MOPC315 (marked by stars in Figures 1 and 2), cannot be explained by sequencing errors. Each of the two Vλ sequences from MOPC315 was obtained from two independent phage clones. Thus we believe these nucleotide substitutions are true mutations in unrearranged Vλ 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 Jλ segments of MOPC315. Differences were found with the published sequences of Jλ2, Jλ3 and Jλ4 (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

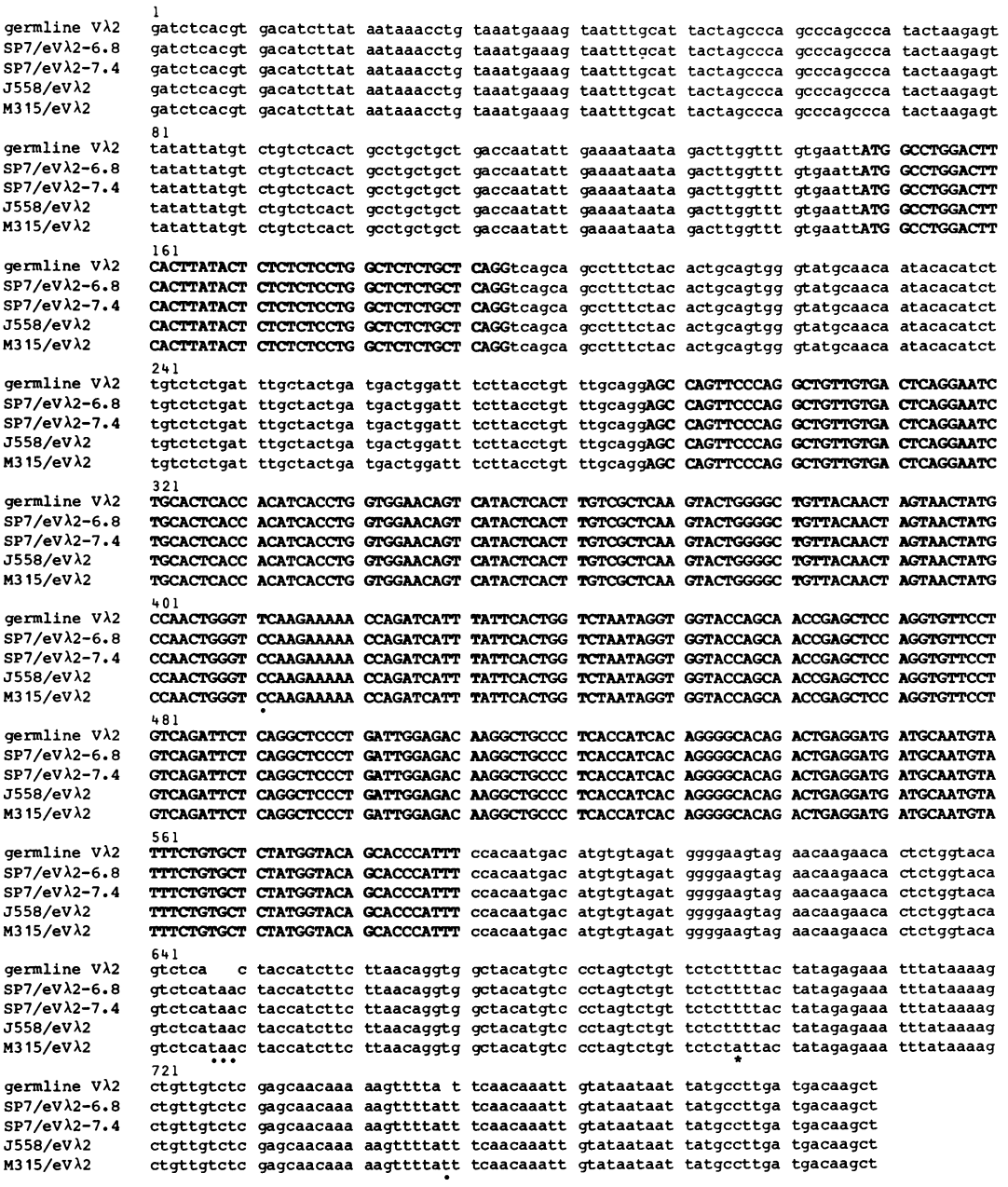

Fig. 2. Comparison of the published sequence of germline Vλ2 with unrearranged Vλ2 gene segments from Sp7 (derived from the fusion partner P3X63Ag8 — see text), J558 and MOPC315. Symbols are used as in Figure 1. The difference at position 411 does not change the assumed germline amino acid sequence.

that the differences were due to errors in the published sequences. Figure 3 displays the sequences of the Jλs from MOPC315 and the unpublished germline sequences of unrearranged Jλ segments.

Can the mutations in the unrearranged Vλ segments be explained by a generally higher mutation rate of MOPC315? To address this question we sequenced four nonrearranged Cλ gene segments. As shown in Figure 4, no differences between the sequences of MOPC315 and the published germline sequences were found for Cλ1, Cλ2 and Cλ3 (Selsing *et al.*, 1982). Our sequence of Cλ4 from MOPC315 contained some differences from the published germline sequence. But cloning and sequencing of Cλ4 from J558, showed it to be identical to Cλ4 from MOPC315. Thus, we conclude, that the four Jλ and Cλ 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 Vκ 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 Vλ segments, a total of three nucleotide substitutions were found in the two unrearranged Vλ segments from MOPC315.

```
                    1
germline Jλ1        ggatcctggg aagaaggatc tttcagtgat gtcaccacct tccaagaatt accaggagct gcatacatca cagatgcaac
M315/eJλ1           ggatcctggg aagaaggatc tttcagtgat gtcaccacct tccaagaatt accaggagct gcatacatca cagatgcaac
                   81
germline Jλ1        ttgagaataa aatgcatgca aggtttttttg catgagtcta tatcacagtg cTGGGTGTTC GGTGGAGGAA CCAAACTGAC
M315/eJλ1           ttgagaataa aatgcatgca aggtttttttg catgagtcta tatcacagtg cTGGGTGTTC GGTGGAGGAA CCAAACTGAC
                  161
germline Jλ1        TGTCCTAGGT gagtgactcc ttcctccttt gttattgttc tctccaagac ttgaggtgct ttttgttgta tactttccct
M315/eJλ1           TGTCCTAGGT gagtgactcc ttcctccttt gttattgttc tctccaagac ttgaggtgct ttttgttgta tactttccct
                  241
germline Jλ1        ttctgtattc tgcttcatac ctatacttca cactaggtaa agaatttctt tcttctctag a
M315/eJλ1           ttctgtattc tgcttcatac ctatacttca cactaggtaa agaatttctt tcttctctag a



                    1
germline Jλ2        taccacccac tgcttctcaa gtgaggtcat agctccaccc attgtagcta gctagtagtt tgat cagct cagctgtgag
M315/eJλ2           taccacccac tgcttctcaa gtgaggtcat agctccaccc attgtagcta gctagtagtt tgattcagct cagctgtgag
                                                                                    •
                   81
germline Jλ2        agaacaggac caggtgctgg ccccataggt tttgggttgg gttttagtca ttgtgtTATG TTTTCGGCGG TGGAACCAAG
M315/eJλ2           agaacaggac caggtgctgg ccccataggt tttgggttgg gttttagtca ttgtgtTATG TTTTCGGCGG TGGAACCAAG
                  161
germline Jλ2        GTCACTGTCC TAGGTaagta gtttcaaagc tt
M315/eJλ2           GTCACTGTCC TAGGTaagta gtttcaaagc tt



                    1
germline Jλ3        cacccacttc aagtgaggtc acagctccac ccattgtagc tagctagtag tttgattcag tgcagctgtg agagaacagg
M315/eJλ3           cacccacttc aagtgaggtc acagctccac ccattgtagc tagctagtag tttgattcag tgcagctgtg agagaacagg
                   81
germline Jλ3        cccaggtgct tgccccacag gtttagggtt gggtttcagt cactgtggTT TATTTTCGGC AGTGGAACCA AGGTCACTGT
M315/eJλ3           cccaggtgct tgccccacag gtttagggtt gggtttcagt cactgtggTT TATTTTCGGC AGTGGAACCA AGGTCACTGT
J558/Vλ1Jλ3                                                        TT TATTTTCGGC AGTGGAACCA AGGTCACTGT
                  161
germline Jλ3        CCTAGGTaag tggctttaat gcttcttcct aataagtcta ggccttgtta tcttgcaagg gtcatttatc tctctctgga
M315/eJλ3           CCTAGGTaag tggctttaat gcttcttcct aataagtcca ggccttgtta tcttgcaagg gtcatttatc tctct  gga
J558/Vλ1Jλ3         CCTAGGTaag tggctttaat gcttcttcct aataagtcca ggccttgtta tcttgcaagg gtcatttatc tctct  gga
                                                      •                             ••



                    1
germline Jλ4        gaatattaca gtgatgtcac cacca ccta ggatcacccc cacctacaca cacagatgca actggaaaat aaggtacatg
M315/eJλ4           ggatattaca gtgatgtcac caccatccta ggatcacccc cacctacaca cacagatgca actggaaaat aaggtacatg
                    •                     •
                   81
germline Jλ4        cagagttttt tgcattagac tatatcagtg tTGGGTGTTC GGAGGTGGAA CCAGATTGAC TGTCCTAGAT gagtgactcc
M315/eJλ4           cagagttttt tgcattagac tatatcagtg tTGGGTGTTC GGAGGTGGAA CCAGATTGAC TGTCCTAGAT gagtgactcc
                  161
germline Jλ4        tccctccttt gttattattg tctccaagct t
M315/eJλ4           tccctccttt gttattattg tctccaagct t
```

Fig. 3. Comparison of the published sequences of germlin Jλ1−Jλ4 with the unrearranged Jλ1−Jλ4 from MOPC315 and the Jλ3 part of the aberrantly rearranged λ3 gene from J558 (Vλ1Jλ3 — see Figure 1). The J558 λ3 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 Vλ segments in MOPC315 are located on different chromosomal homologues (Hozumi *et al.*, 1982), it is possible that either Vλ1 or Vλ2 is derived from C57BL/6. The coding region of Vλ1 in both strains is identical (Bothwell *et al.*, 1982). Since the 3′ flanking region of Vλ2 from C57BL/6 has not been sequenced the two strains might be different in that region. The chance that the 3′ flanking region of Vλ2 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 Jλ segments of MOPC315. None were found in the four Jλ segments we sequenced. We also found no mutations in four different unrearranged Cλ 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 Vλ 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

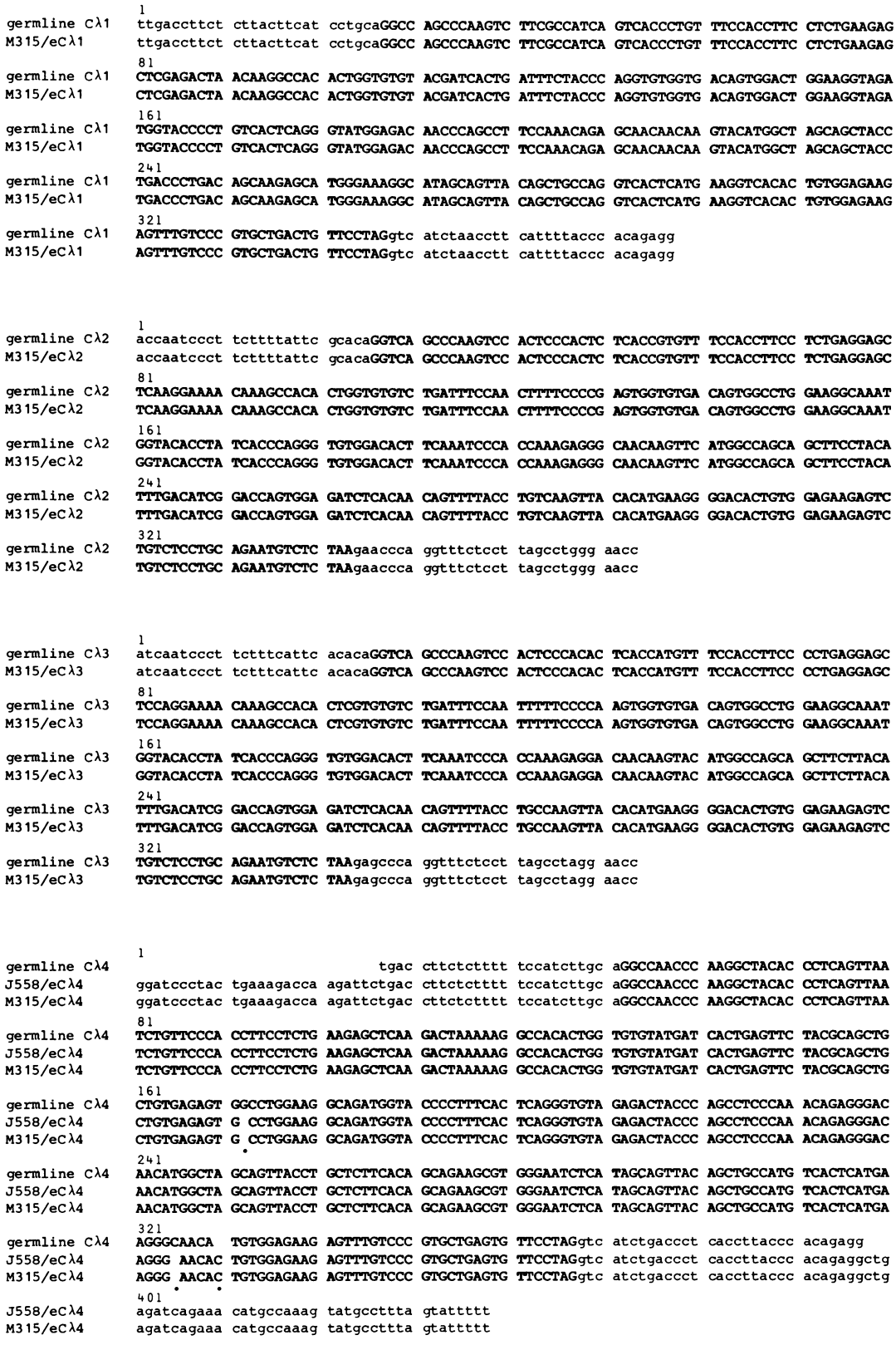

Fig. 4. Comparison of published sequences of germline Cλ1 − Cλ4 with unrearranged Cλ1 − Cλ4 from MOPC315 and Cλ4 from J558. The dots indicate differences in our sequences from the published ones.

of somatic mutations in the rearranged λ 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 Vλs are certainly separated by >12 kb from the Cλ clusters (Selsing *et al.*, 1982) while the JλCλ introns are only ~1.2 kb long. It is more reasonable to assume that recognition sites are in or near the Vλ 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 λgtwes·λB, 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.

References

Bernard,O., Hozumi,N. and Tonegawa,S. (1978) *Cell*, **15**, 1133-1144.
Blomberg,B. and Tonegawa,S. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 530-533.
Bothwell,A.L.M., Paskind,M., Reth,M., Imanishi-Kari,T., Rajewsky,K. and Baltimore,D. (1981a) *Cell*, **24**, 625-637.
Bothwell,A.L.M., Paskind,M., Schwartz,R.C., Sonenshein,G.E., Gefter,M.L. and Baltimore,D. (1981b) *Nature*, **290**, 65-67.
Bothwell,A.L.M., Paskind,M., Reth,M., Imanishi-Kari,T., Rajewsky,K. and Baltimore,D. (1982) *Nature*, **298**, 380-382.
Carè,A., Cianetti,L., Giampaola,A., Sposi,N.M., Zappavigna,V., Mavilio,F., Alimena,G., Amadori,S., Mandelli,F. and Peschle,C. (1986) *EMBO J.*, **5**, 905-911.
Gorski,J., Rollini,P. and Mach,B. (1983) *Science*, **220**, 1179-1181.
Hamlyn,P.H., Gait,M.J. and Milstein,C. (1981) *Nucleic Acid Res.*, **9**, 4485-4494.
Hozumi,N., Wu,G.E., Murialdo,H., Roberts,L., Vetter,D., Fife,W.L., Whiteley,M. and Sadowski,P. (1981) *Proc. Natl. Acad. Sci. USA*, **11**, 7019-7023.
Hozumi,N., Wu,G., Murialdo,H., Baumal,R., Mosmann,T., Winberry,L. and Marks,A. (1982) *J. Immunol.*, **129**, 260-266.
Kearney,J.F., Radbruch,A., Liesegang,B. and Rajewsky,K. (1979) *J. Immunol.*, **123**, 1548-1550.
Köhler,G. and Milstein,C. (1976) *Eur. J. Immunol.*, **6**, 511-519.
Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning. A Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York.
Manser,T., Wysoki,L.J., Gridley,T., Near,R.I. and Gefter,M.L. (1985) *Immunol. Today*, **6**, 94-101.
McKean,D., Huppi,K., Bell,M., Staudt,L., Gerhardt,W. and Weigert,M. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 3180-3184.
Miller,J., Selsing,E. and Storb,U. (1982) *Nature*, **295**, 428-430.
Nishioka,Y. and Leder,P. (1980) *J. Biol. Chem.*, **255**, 3691-3694.
Pech,M., Höchtl,J., Schnell,H., and Zachau,H.G. (1981) *Nature*, **291**, 668-670.
Rabbits,T.H., Hamlyn,P.H. and Baer,R. (1983) *Nature*, **306**, 760-765.
Sablitzky,F., Wildner,G. and Rajewsky,K. (1985) *EMBO J.*, **4**, 345-350.
Schilling,J., Clevinger,B., Davie,J.M. and Hood,L. (1980) *Nature*, **283**, 35-40.
Selsing,E. and Storb,U. (1981) *Cell*, **25**, 47-58.
Selsing,E., Miller,J., Wilson,R. and Storb,U. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 4681-4685.
Showe,L.C., Ballantine,M., Nishikura,K., Erikson,J., Kaji,H. and Croce,C.M. (1985) *Mol. Cell. Biol.*, **5**, 501-509.
Sirisinha,S. and Eisen,H. (1971) *Proc. Natl. Acad. Sci. USA*, **68**, 3130-3135.
Smith,A.J.H. (1980) *Methods Enzymol.*, **65**, 560-580.
Tonegawa,S. (1976) *Proc. Natl. Acad. Sci. USA*, **73**, 203-207.
Tonegawa,S. (1983) *Nature*, **302**, 575-581.
Tonegawa,S., Maxam,A.M., Tizard,R., Bernard,O. and Gilbert,W. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 1485-1489.
Wabl,M., Burrows,P.D., von Gabain,A. and Steinberg,C. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 479-482.
Walfield,A., Selsing,E., Arp,B. and Storb,U. (1981) *Nucleic Acid Res.*, **9**, 1101-1109.
Weigert,M.G., Cesari,I.M., Yonkovich,S.J. and Cohn,M. (1970) *Nature*, **228**, 1045-1047.
Weiss,S., Meyer,J. and Wabl,M.R. (1985) *Eur. J. Immunol.*, **15**, 765-768.
Wu,G.E., Govindji,N., Hozumi,N. and Murialdo,H. (1982) *Nucleic Acid Res.*, **10**, 3831-3843.
Yancopoulos,G.D. and Alt,F.W. (1986) *Annu. Rev. Immunol.*, **4**, 339-368.
Yanisch-Perron,C., Vieira,J. and Messing,J. (1985) *Gene*, **33**, 103-119.

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