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Nucleotide sequence of a chromosomal rearranged λ_2 immunoglobulin gene of mouse

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ABSTRACT

The rearranged λ_2 gene of the mouse plasmacytoma cell line MOPC315 has been cloned and sequenced. A comparison of its sequence with the sequence of the unrearranged (germ-line) V, J and C gene segments shows that the sequences of the V gene segments differ at six positions. The sequence of the J and C gene segments remained unchanged. These results add support to the hypothesis that somatic mutations occur in immunoglobulin genes and that these mutations do not involve the C gene segment. The degree of homology of the elements of the λ_2 gene with those of the λ_1 gene and $C\lambda_3$ and $C\lambda_4$ gene fragments suggest a pathway of evolution by gene duplication of the immunoglobulin λ light chain locus. According to this scheme the original structure V_0-J_0C_0 gave rise to a structure V_0-J_1C_1-$J_{11}C_{11}$ by duplication of the J_0C_0 region. A second duplication encompassing the whole region resulted in the present structure: V_1-J_3C_3-J_1C_1/V_2-J_2C_2-J_4C_4.

INTRODUCTION

The genetic analysis of antibody gene organization has suggested several possible mechanisms for the generation of V sequence variation in the polypeptides of the antibody molecule. One mechanism consists of the random recombination of one of the many V gene segments with one of the J gene segments for the light chains or with D and J gene segments for the heavy chain (1, 2). Furthermore this recombination is flexible so that some variation is found in the cross-over point in V-J, V-D and D-J recombination (3-6).

The second mechanism is somatic mutation. This has been found by the study of the amino acid sequences of myeloma proteins and the nucleotide sequence of unrearranged versus rearranged V and J gene segments (7).

Indeed, one of the first cases in which the DNA sequence of the unrearranged and rearranged V gene segment was analysed showed that the two sequences were not identical (8). Since then, differences have been found in the nucleotide sequence of unrearranged and rearranged J gene segments (9, 10) as well as in a series of V gene segments of the (11, 12)

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and heavy chains (13, 14).

In order to conclude that a V gene segment underwent somatic mutations in its pathway from the unrearranged germ-line to the rearranged configuration one must make absolutely certain that one is dealing with the same gene, and not with another germ-line V gene segment of the same group. Thus, in all cases in which the κ light chain has been the subject of study it was necessary to clearly prove that the identified germ-line V gene segment was indeed the only possible ancestor of the rearranged gene in question. This problem does not arise in situations in which there is ample evidence that only one V gene segment exists. Such is the case of the λ light chains (15). Thus the finding that the V sequence of the rearranged λ_1 gene of HOPC2020 differed from its germ-line counterpart is unequivocal evidence for somatic mutation (8). In this paper we present the sequence of the rearranged λ_2 gene of the myeloma cell line MOPC315 and show that its V gene segment also differs from the corresponding germ-line sequence.

MATERIALS AND METHODS

MOPC315 is an IgA, λ_2 secreting myeloma adapted to grow *in vitro*. MOPC315.26 is a derivative of MOPC315 which secretes the λ_2 chain but synthesizes no heavy chain.

E. coli 803 is an r_K^- m_K^-, *supF* strain. It was used as a plating indicator and for the growth of phage stocks. *E. coli* NS428 was used as a source of extract for packaging of phage λ DNA *in vitro*. The molecular vector used was λgtWES-λB.

The probes used in Southern blotting (16) and phage plaque hybridization (17) were prepared from two pBR322 derived plasmids containing cDNA's of λ_1 and λ_2 mRNA's. Plasmid HOPC-λ20, a gift of J. Adams, contains cDNA corresponding to HOPC1 myeloma λ_1 mRNA and plasmid pλ2-1 (18), a gift of G. Sonenshein, contains cDNA corresponding to MOPC315 myeloma λ_2 mRNA. The probes were prepared by nick-translation of a 300 bp *Kpn*1 fragment of HOPC-20 (λ_1VJC probe) and a 580 bp *Pst*I-*Bam*HI fragment of pλ2-1 (λ_2VJC probe). The fragments contained part of V, J and part of C sequences (fig. 1).

The origin of the cell lines, bacterial and phage strains as well as the culture conditions and media used has already been described (19). The procedures for phage growth, DNA isolation, nick-translation, nitrocellulose blotting, molecular cloning, isolation of fragments for DNA sequencing and the sequencing procedures (20) and the electron microscopic analysis of heteroduplexes of DNA were carried out as described (19).

RESULTS

Genomic Arrangement of MOPC315.26 λ chain genes:

In order to determine the arrangement of MOPC315.26 λ chain genes an EcoRI digest of cellular DNA was analysed by nitrocellulose blotting (16) using the λ_1VJC probe (fig. 1). The V sequences in this probe are able to hybridize to Vλ_1 and Vλ_2 since there are only 12 bp differences between the two V gene segments (8). On the other hand a difference of about 25 amino acids between the C region of the λ_1 and λ_2 chains (21, 22) precludes cross-hybridization of the λ_1VJC probe with Cλ_2 sequences. The results are shown in figure 1. EcoRI digested mouse kidney DNA which was used as a reference contained three bands of 8.6 kb, 4.8 kb and 3.5 kb. These bands have been shown to contain the germ-line sequences coding for Cλ_1, Vλ_2 and Vλ_1 respectively (6, 15, 18, 23). The restricted DNA of MOPC315.26 cells showed in addition to the above three bands, two bands of 7.4 kb and 6.6 kb. The 7.4 kb band corresponds to an aberrantly rearranged λ_1 chain gene (19), and since the 6.6 kb is the only other band present in the DNA of this λ_2 chain producer, it had been tentatively identified as a rearranged λ_2 chain gene (24). We therefore cloned the DNA in this region of a preparative gel into λgtWES and the recombinant phages were screened for λ_2 sequences using the procedure of Benton and Davis (17) with the λ_1VJC radioactive probe. Several clones gave positive signals and one of them named λgtWES-λ_2.315.26C, (λgt-$\lambda_2$26), was characterized further.

DNA Heteroduplex Analysis of the Cloned λ_2 6.6 kb Fragment:

To corroborate that the 6.6 kb EcoRI insert in λgt-$\lambda_2$26 was indeed a rearranged λ_2 chain gene, the phage DNA was digested with EcoRI and the product of the digestion was hybridized with EcoRI-linearized pλ2-1, which is pBR322 containing a cDNA of λ_2 mRNA inserted at the PstI site. The heteroduplexes formed were observed under the electron microscope and one example is shown in fig. 2. It can be seen that there is a small region of about 700 bp long which hybridizes between the 6.6 kb insert and the linearized plasmid. This stretch of homology is interrupted in the middle, by a single strand about 1200 bp long. This loop most likely corresponds to the intron, present in the genomic DNA but absent in the cDNA, and the two flanking regions that form duplex DNA must correspond to the VJ and C genes. The ability of the 6.6 kb EcoRI insert to form such type of heteroduplexes with λ_2 cDNA proves that indeed it contains a λ_2 rearranged gene.

The degree of homology between the DNA sequences of rearranged λ_2 and λ_1 genes, was analysed in DNA heteroduplexes formed between the cloned λ_2

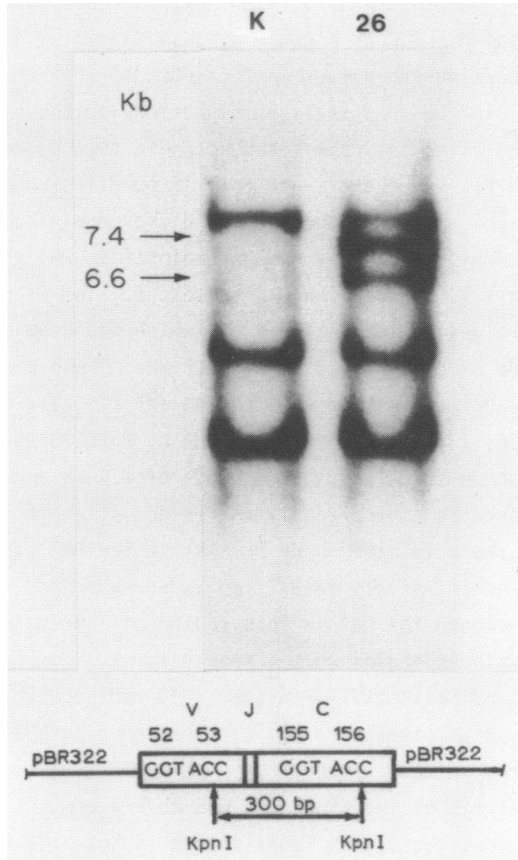


Figure 1:
Analytical Southern blot of DNA from MOPC315.26 and mouse tissue. EcoRI-digested DNA (20 μg) was fractionated through a 1% agarose gel for the Southern blot. Lane K contained kidney DNA and the lane 26 contained DNA from MOPC315.26. The three unlabelled bands correspond respectively to unrearranged $C\lambda_1$ (8.6 kb), unrearranged $V\lambda_2$ (4.8 kb), and unrearranged $V\lambda_1$ (3.5 kb) (6, 15, 18, 23). The probe used, a 300 bp KpnI fragment of λ_1 cDNA, is shown at the bottom of the figure and corresponds to the λ_1 VJC probe.

gene and the rearranged λ_1 gene of the HOPC2020 myeloma also cloned in λgtWES (19). Figure 3 shows two different classes of heteroduplexes. The type of heteroduplex shown in panel A constituted about 80% of the molecules. Twenty percent of the heteroduplex were like the one shown in panel B. The regions of homologies outside the heterologous regions correspond to the arms of λgtWES. Between the arms, starting at the bottom, there are single strands of 5.8 kb and 2.8 kb, in the case of the molecule in panel A, which

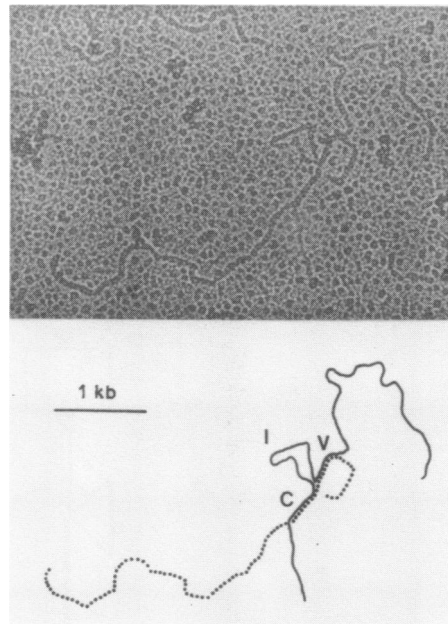


Figure 2:
Heteroduplex of the 6.6 kb λ_2 fragment and λ_2 cDNA. The λ_2 cDNA was inserted at the PstI site of pBR322, which was linearized with EcoRI. In the interpretative drawing, the continuous and dotted lines represent single-stranded DNAs of the 6.6 kb genomic fragment and of the plasmid containing the λ_2 cDNA, respectively. V, I and C indicate the position of the V gene fragment, the intron and gene C, respectively.

represent the region of nonhomology downstream from the VJ genes of λ_1 and λ_2 respectively. In the molecule in panel B, however, a region of homology of about 0.4 kb shows up in the middle of this region of single strands. This represents the C gene of λ_1 and λ_2 which have been shown to be only partially homologous. The region of 1.2 kb between C and V in the molecule in panel B represents the heterologous introns of λ_1 and λ_2. The next 2.0 kb of homologous region represents 0.4 kb of V regions and 1.6 kb upstream of the V's. This region of 1.6 kb upstream of V is not completely homologous in the two rearranged genes since although in the great majority of the heteroduplexes observed it showed as duplex, occasionally a few molecules were observed in which only the VJ gene had hybridized (not shown). The 1.7 kb single stranded loop at this point represents the remainder of the DNA that is present in the fragment containing λ_2.

The fact that in all heteroduplexes observed the VJ gene was in duplex

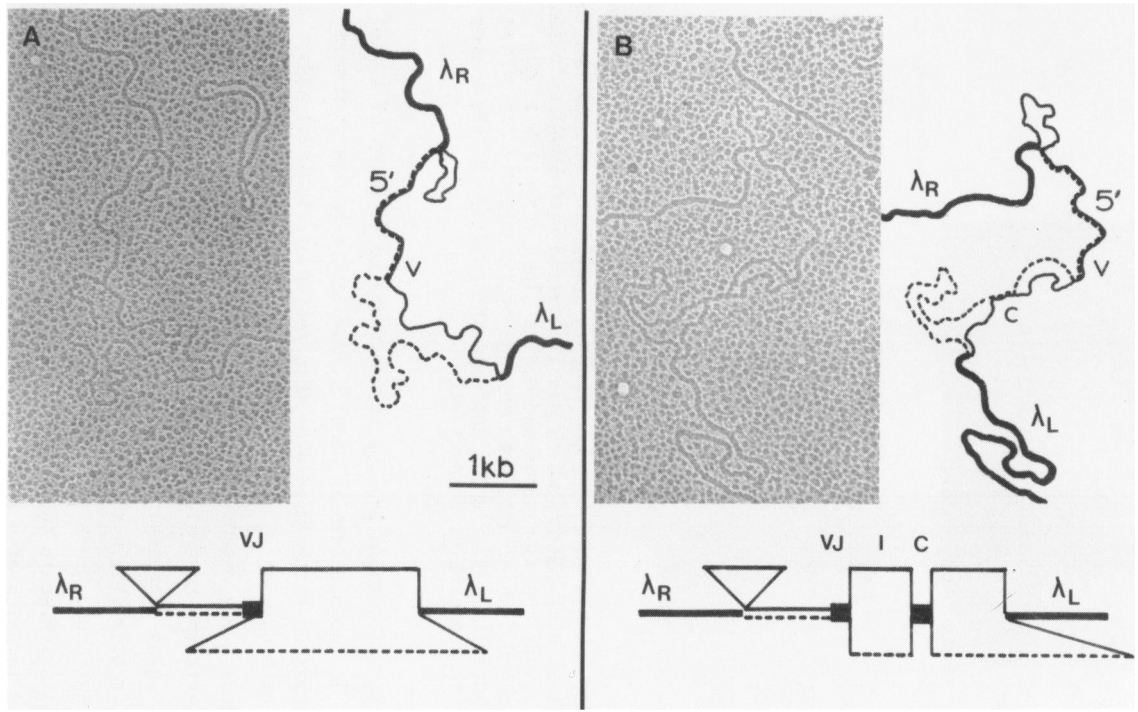


Figure 3:
Heteroduplex of the λgt-$\lambda_2$26 and λgt-λ_1HOPC2020. In the interpretative drawings and in the schemes at the bottom of the figure the arms of phage lambda are shown as thick continuous lines and are labelled λR and λL. The continuous and discontinuous thin lines represent single-stranded DNA of the λ_2-containing 6.6 kb and λ_1-containing 7.4 kb inserts, respectively. When in the form of duplex DNA the VJ and C genes are shown as filled boxes. I stands for intron.

form is in agreement with the known similarities in nucleotide sequence of $V\lambda_1$ and $V\lambda_2$ gene segments. In addition, our results show that 1.6 kb of DNA upstream from the V gene segment must have a rather high sequence homology since 94% of the heteroduplexes observed were duplex in this region. The constant portion of the λ_1 and λ_2 chains differ by about 25 amino acids. However, about 20% of the heteroduplexes observed had the C gene in duplex form. When expressed in terms of sequence homology (see below) the two C genes are still 74% homologous, and this degree of homology still allows hybridization of about 20% of the heteroduplexes under our renaturation conditions. In no case (less than 3%) did we observe hybridization between λ_1 and λ_2 introns, therefore, the degree of homology of the two introns must be rather small.

Restriction Endonuclease Mapping of the $\lambda gt\text{-}\lambda_2 26$:

In order to determine the nucleotide sequence of the λ_2 gene a detailed restriction map was required. This was done in two steps. Firstly, the DNA from $\lambda gt\text{-}\lambda_2 26$ was digested with one or more enzymes that cleave DNA rather infrequently and the digestion products analysed by the Southern technique (16) to identify sequences homologous to a VJC λ_2 probe. The result of this analysis is shown diagramatically in figure 4. We found that the λ_2 structural genes were present on three readily separable fragments. Fragment a, a 950 bp BglII to BglII fragment contained the V gene segment, fragment b, a 750 bp BglII to BglII fragment contained most of the C gene and fragment c, a 1100 bp BglII to EcoRI fragment contained the rest of the C gene. In the second step, these three fragments were isolated by the procedure of Maxam and Gilbert (21) from a preparative 7.5% polyacrylamide gel (25) and a detailed restriction enzyme cleavage map of each fragment was constructed (figure 4).

DNA Sequencing:

The sequencing strategy that we used is shown under the enzyme cleavage sites in figure 4. In general the sequences were determined at least twice in both the coding and non-coding strands. The DNA sequence for the λ_2 structural gene together with the amino acid sequence predicted from the triplet codons is shown in figure 5.

The landmarks in the sequence are as follows: There are two TATA box-like sequences, one TATATT (nucleotide 81-86), and the other, TATAAT (nucleotide 18-23). We do not know which, if either of these sequences are used.

The leader sequence, or signal peptide is 19 codons long and is inter-

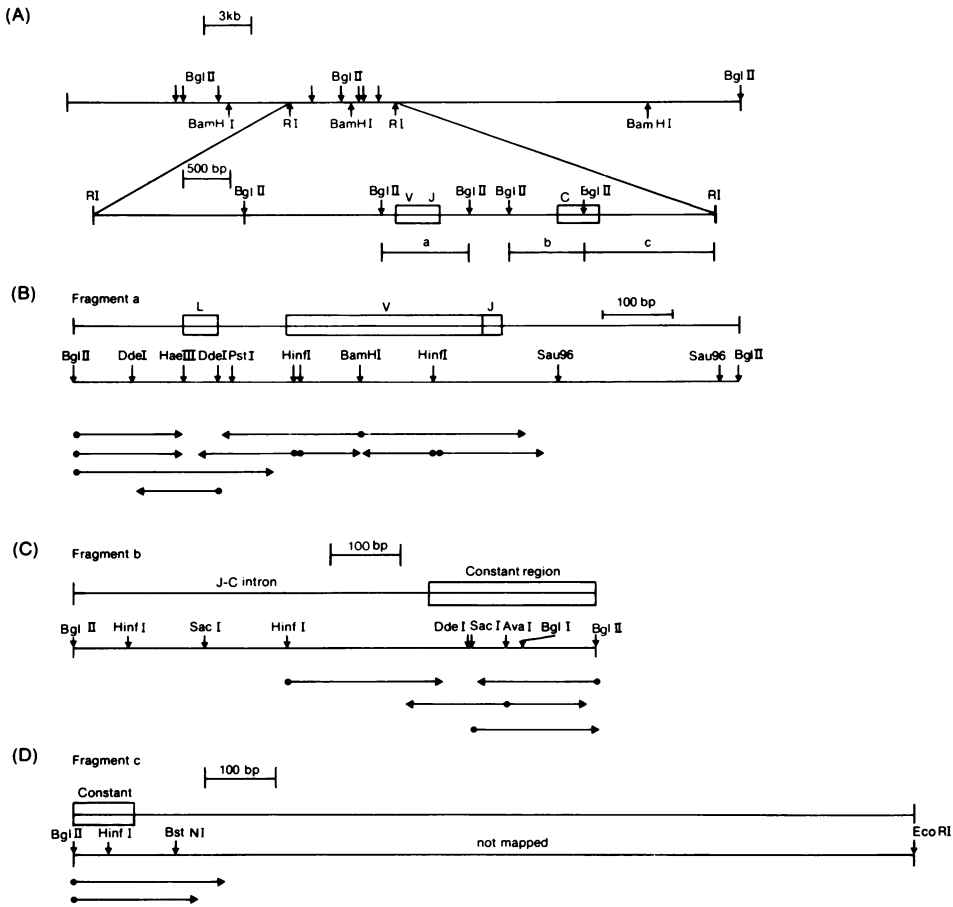
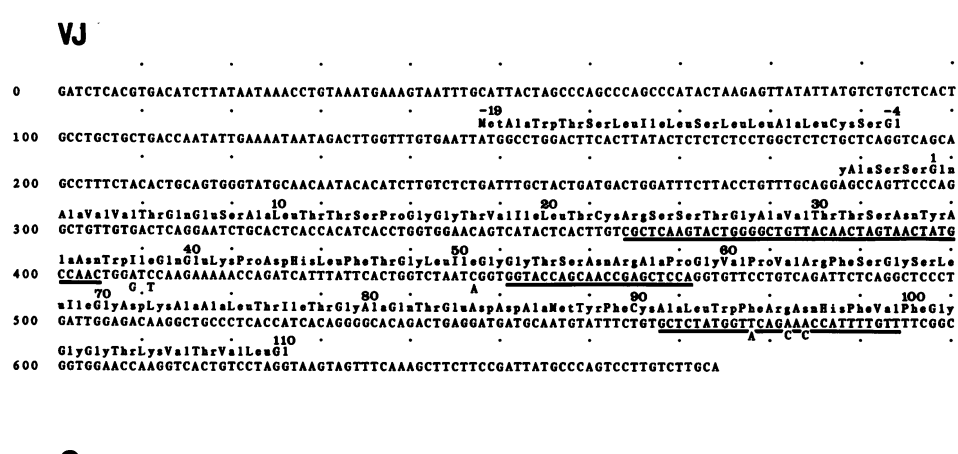


Figure 4:
DNA Restriction map of the λ_2-containing 6.6 kb fragment and DNA sequencing strategy. A: Map of λgt-$\lambda_2$26 showing the left and right arms of the vector phage and the insert of mouse DNA carrying the λ_2 gene. The expanded section corresponds to a more detailed restriction map of the mouse DNA insert. B, C and D show the restriction maps and DNA sequencing strategies of the fragment labelled a, b and c in the map in A.

rupted by a 93 bp intron that splits codon -4. The structural gene consists of 214 codons and is interrupted in codon 110 by an intron which by heteroduplex mapping, we have shown to be about 1200 bp long. The termination codon, 215, is TAA. We see no polyadenylation addition sites within 50 bp downstream from the termination codon, which is not unexpected since the λ_2 mRNA is 1100 bases long (18). The $\underline{C}\lambda_2$ germ-line gene has been sequenced



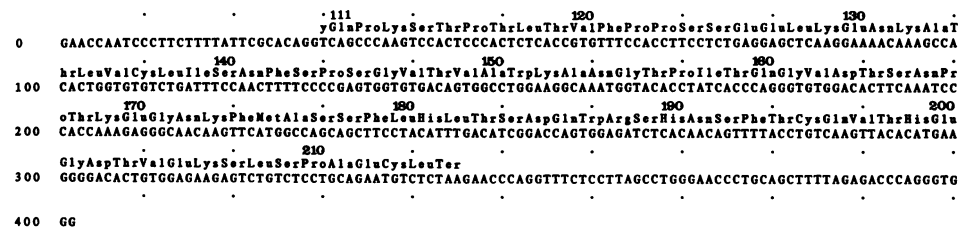


Figure 5:
DNA sequence of VJ and C of the λ_2 gene of MOPC315.26. A: sequence of VJ, B: sequence of C. Only the sense strand is shown. The nucleotides have been numbered sequentially and independently for VJ and C starting at 1 (left margin numbers). The corresponding amino acids are shown above the DNA sequence and are numbered starting at amino acid 1 (Gln), which marks the beginning of the V region. Negative numbers refer to the amino acids of the leader sequence.

by U. Storb et al. (personal communication) and it corresponds exactly with the sequence of the constant gene of the λ_2 rearrangement of MOPC315 determined by us. This fact further substantiates our prediction of the λ_2 protein sequence.

DISCUSSION

In this study we have determined the DNA sequence of the structural gene for the MOPC315λ_2 immunoglobulin light chain.

The amino acid sequence specified by this nucleotide sequence is in close agreement to that determined by sequencing the protein (22, 25). We find amino acid 55 to be Asn rather than Asp, amino acids 145 and 146 to be Gly-Ser rather than Ser-Gly, and amino acid 166 to be Asn rather than

Asx. Furthermore we have determined the order of the previously unordered amino acids 165-168; they are Ser-Asn-Pro-Thr. These changes reflect the difficulties in determining the protein sequences and we feel do not indicate a real difference in the two sequences. The validity of this supposition is increased upon examination of the nucleotide sequence of the germ-line $\underline{V}\lambda_2$ (26) and $\underline{C}\lambda_2$ (U. Storb, personal communication) genes at these codons. The germ-line genes have the same sequence at these codons as the λ_2 rearranged gene.

Somatic Changes Are Present in the Variable Region of the λ_2 Gene:

The sequence of the germ-line gene for the variable region of λ_2 light chain has been reported by Tonegawa et al. (26). If somatic mutation plays a role in the generation of antibody diversity we might expect that the rearranged gene sequence would differ from that of the germ-line. In figure 5 a comparison of the nucleotide sequence of the rearranged λ_2 gene of MOPC315 determined here, with the sequence of the unrearranged $V\lambda_2$ (26), $J\lambda_2$ (23, 27) and $C\lambda_2$ (U. Storb, personal communication) is shown. We found that the rearranged λ_2 gene differed from its unrearranged counterparts at six nucleotide positions all of which occurred in the V gene segment. There were no mutations present in the 3' side of the V gene segment. There are three changes in the second framework region resulting in one substitution at amino acid number 38 (GTT-Val to ATC-Ile) and one silent change at amino acid number 50 (ATA-Ile to ATC-Ile). In the third variable region, there are three nucleotide changes all of which result in amino acid changes. At position 94, a change from TAC to TTC results in Phe being substituted by Tyr; at position 95, a change from AGC to AGA results in Ser being substituted by Arg, and at position 96, a change from ACC to AAC results in Thr being substituted by Asn. Thus, of the eight amino acids of this hypervariable region, three have been changed.

Since three of the base changes are in the hypervariable regions and three are in the framework regions, somatic mutations do not seem to occur preferentially in any particular region of the V gene segment. These results are in agreement with the conclusions of other workers (5, 6, 8, 11, 12, 31, 32). Although the distribution of the mutations in the λ_2 gene of MOPC315 appear to be in two clusters, a survey of mutations in several other rearranged genes (5, 6, 8, 11, 12, 31, 32) showed no cluster and no obvious polarity in the frequency of mutations. The finding that the $\underline{C}\lambda_2$ sequence of MOPC315 is identical to the germ-line $\underline{C}\lambda_2$ adds to the increasing evidence that C regions do not undergo (silent) somatic mutations; indeed, the $\underline{C}\kappa$

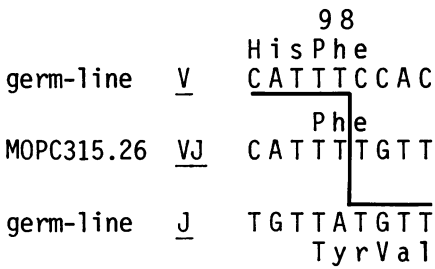


Figure 6:
Site of V-J recombination in the λ_2 of MOPC315.26.

gene has been sequenced twice in germ-line configuration (28, 29) and once in rearranged configuration (29) and all three sequences are identical. A comparison of the nucleotide sequence of the unrearranged $V\lambda_2$ (26) and $J\lambda_2$ (23, 27) with the rearranged λ_2 allows us to deduce unambiguously the exact point at which the cross-over took place in the generation of the rearranged λ_2 gene of MOPC315. This was in codon 98 as illustrated in figure 6.

Homology Between the λ_1 and λ_2 Genes:

The germline $V\lambda_1$ and $V\lambda_2$ gene segments have been sequenced and found to differ in only 12 nucleotides (8, 26). Furthermore 1.6 kb of DNA upstream from the $V\lambda_1$ and $V\lambda_2$ gene segments forms duplex DNA readily as shown in figure 3. This suggests that these two genes arose by a recent gene duplication. Although the high degree of homology between the coding regions could be due to some functional constraint exerted on the gene products we cannot easily explain the extended homology in the region upstream of V on the same basis. The $C\lambda_1$ germline gene has been sequenced from positions corresponding to amino acid 110 to 209, (ref. 8 and Hozumi, unpublished results) and within these 300 bp there are 79 differences with the nucleotide sequence of $C\lambda_2$ from MOPC315 (or germline). In addition, the sequences upstream from C, that is the J-C intron, and the sequences downstream from C have very little homology as evidenced from the heteroduplex analysis of rearranged λ_1 and λ_2 genes (figure 3). Thus, in contrast to the V gene segment, $C\lambda_1$ and $C\lambda_2$ have a much longer history of independent evolution.

Blomberg and Tonegawa (30) have discussed the evolutionary origin of the λ family of genes in mouse based on the nucleotide sequence of the four J gene segments and their neighbouring regions. Based on this data they proposed that $C\lambda_2$ and $C\lambda_3$ diverged from each other more recently than the putative ancestors $C\lambda_{II}$ and $C\lambda_I$. The first of these ancestors is supposed to have given origin to $C\lambda_2$ and $C\lambda_4$ and the latter to $C\lambda_3$ and $C\lambda_1$. Thus, $C\lambda_2$ nucleotide sequence should be more similar to $C\lambda_3$ than to $C\lambda_1$. Indeed,

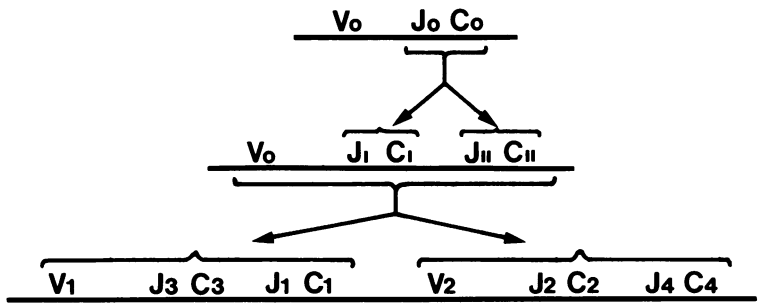


Figure 7:
Putative pathway of evolution by gene duplication of immunoglobulin λ light chain locus of mus musculus.

$C\lambda_2$ is only 74% homologous to $C\lambda_1$ whereas it is 97% homologous within the coding region and 91% in 56 nucleotides flanking the coding region to $C\lambda_3$ (U. Storb, personal communication).

The close resemblance in nucleotide sequence of $V\lambda_1$ with $V\lambda_2$ and their flanking regions and of $C\lambda_2$ with $C\lambda_3$ suggests that the duplication that created these genes from a common ancestor took place about the same time in evolution, and that indeed, it could have been the same event. A scheme showing how this could have happened is shown in figure 7.

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