ORGANIZATION AND EXPRESSION OF MOUSE λ LIGHT CHAIN IMMUNOGLOBULIN GENES [1]

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ABSTRACT The four λ light chain constant region (C) genes have been cloned from BALB/c mouse embryo DNA. The $C\lambda_1$ gene segment was previously analyzed (1,2). Each Cλ gene carries its own J segment approximately 1.3 kilobases to its 5' side which contrasts with both the kappa (κ) and heavy (H) chain immunoglobulin gene systems with a cluster of four functional joining (J) sequences 5' to the constant gene segment(s). The four Cλ genes occur in two clusters: $5'J_3C_3J_1C_1 3'$ and $5'J_2C_2J_4C_4 3'$. The J DNA segments of λ_2, λ_3 and λ_4 were sequenced and compared with that of λ_1. Sequence homology (particularly in the non-coding regions) was greatest between J_1 and J_4 and between J_2 and J_3 which suggests, along with the similar organization of JCJC and crosshybridization of C_1 and C_4 and of C_2 and C_3, that the two clusters are products of a duplication event. A single variable region (V)λ gene, 5' of each JCJC cluster, was probably part of this duplication unit. We have confirmed that there are only two Vλ genes in mouse ($V\lambda_1$ and $V\lambda_2$), and we have also shown that the $V\lambda_1$ gene segment is joined productively to $C\lambda_3$ in a λ_3 myeloma. $V\lambda_1$ has been found associated only with $C\lambda_3$ or $C\lambda_1$ and in most cases $V\lambda_2$ joins with $C\lambda_2$ (the exceptions allow us to deduce a probable organization of the total λ locus). From these data and from the analysis of germ line and rearranged Vλ genes in myelomas, the two Vλ genes must be interspersed by a JCJC cluster if the looping-out and deletion model is generally used for V-J joining. The organization of the λ locus is most

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likely: 5'V_2-$J_2C_2J_4C_4$-V_1-$J_3C_3J_1C_1$3'. The λ_4 gene is probably not functional since the J_4 sequence does not contain a valid splice site and has a 2-bp deletion in the signal heptamer sequence 5' to J_4. The signal nonamer sequence 5' to J_3 differs from that of J_1 in two consecutive base pairs and may account for the lower level of λ_3 expression as compared with λ_1 in mouse lymphocytes.

INTRODUCTION

The immunoglobulin genes for the light chains, κ and λ, and the heavy chains occur in three families. In mouse, the genes for the light chains have been placed on chromosome 6 (3,4), the heavy (H) chains on chromosome 12 (5,6) and the light chains on chromosome 16 (7). It was proposed that the λ germ line variable (V) region gene segments are separate from the constant (C) gene segments for light chains (8) and likewise for heavy chains (9, 10). This assumption was demonstrated to be correct and it was found that formation of a functional immunoglobulin (Ig) gene requires DNA linkage of V to the J (joining) region in the case of light chain genes (11, 1) and V, D, and J in the case of heavy chain genes (12, 13)(the D, diversity, DNA segments encode primarily the third hypervariable region of heavy chains). For the κ light chain, one of about two hundred V genes (14, 15) can combine with one of four functional J segments (16, 17). The exact site of joining may vary slightly (16, 17). Thus two somatic mechanisms occur to generate diversity within a light chain: combinatorial diversity when different combinations of V and J gene segments assemble and junctional diversity when different amino acids are generated by slippage in the actual site of V-J joining. Likewise, increased antibody diversity is generated when one of the V_H gene segments is joined with one of approximately twenty D segments (18) and one of four J_H segments (13, 12). Junctional diversity can also occur for heavy chains (13, 19). Antibody diversity is also generated when different light chains combine with different heavy chains. The antibody repertoire is further expanded by somatic mutation of the V gene segments for both light chains (20, 2, 21, 22) and heavy chains (13, 23-26).

We wished to study the organization and molecular basis for differential expression of the mouse λ light chain genes. The mouse λ light chain system is simple when compared with that of the κ and H chains in that there are only two V genes, V_1 and V_2, (1) and the λ myeloma and serum proteins have been well defined. The λ light chains, which comprise about 5% of the mouse serum Ig (27-29) occur in three subtypes, λ_1 (20, 30, 31), λ_2 (32, 33), and λ_3 (34). A subtype is defined by

its C region amino acid sequence. The three subtypes, λ_1, λ_2 and λ_3 occur in the serum in the approximate ratio 8:1:1 (29, 34) and in spleen lymphocytes in the approximate ratio 1.0: 0.7: 0.3 (35). The λ_1 chains previously reported all contained V regions encoded by V_1 (1, 15) and all known λ_2 chains use V_2 (29, 34, 36). It was recently shown that λ_3 chains also use the V_1 gene (37,38). Each of the subtypes is encoded by its own constant region gene. We describe here the V, J, and C gene segments of the mouse λ locus and give their probable organization in the genome. We show the DNA sequences surrounding each of the λJ segments, suggest a mechanism for control of λ_1 and λ_3 subtype expression, and demonstrate that λ_4 is a pseudogene.

RESULTS AND DISCUSSION

Description and Characterization of Four λC Genes. High molecular weight kidney DNA from several mouse strains was digested with EcoRI endonuclease and analyzed by the Southern gel blotting procedure as previously described (37). Six mouse strains gave identical results as those shown for the BALB/c mouse strain in Figure 1A. The hybridization probe was either $C\lambda_1$ cDNA (600 base pairs complementary to $C\lambda_1$ from an Hha I/Hae III digest of the B1 plasmid of H2020 cDNA $(V+C)\lambda_1$) or $(V+C)\lambda_2$ cDNA (cDNA prepared from MOPC 315, containing $(V+C)\lambda_2$, from R. Schwartz and M. Gefter). With the $(V+C)\lambda_2$ probe we usually saw four bands, at 8.6, 4.8, 3.5, and 3.2 kilobases (kb). The 4.8 kb and 3.5 kb bands are $V\lambda_2$ and $V\lambda_1$ respectively (the two Vλ genes cross hybridize) (1). The 8.6 kb and 3.2 kb bands were candidates for the $C\lambda_2$ and $C\lambda_3$ genes. We expected that $C\lambda_2$ and $C\lambda_3$ would be detected by the $(V+C)\lambda_2$ probe since the amino acid sequences of $C\lambda_2$ and $C\lambda_3$ differ by only five out of 107 residues (34). With the $C\lambda_1$ probe we saw a band at 8.6 kb, known to contain the $C\lambda_1$ gene (1) and another band at 2.8 kb which we designated $C\lambda_4$ (37). The $C\lambda_4$ band did not cross hybridize with the $(V+C)\lambda_2$ probe and was not a candidate for the $C\lambda_2$ or $C\lambda_3$ gene. Occasionally, we detected a band at 6 kb (due to partial digestion, not shown in Figure 1A) which hybridized with both probes.

The bands at 2.8 kb, 6.0 kb, and 8.6 kb were enriched by preparative gel electrophoresis and cloned in λWES phage. A clone of the 8.6 fragment was selected by hybridization with the $(V+C)\lambda_2$ probe but was found to hybridize with both probes and was identical to our previously described clone of $C\lambda_1$, Ig25 (1)(Figure 1B). When the Ig25 cloned DNA is digested with EcoRI, the 8.6 kb band hybridizes to both the $C\lambda_1$ and $(V+C)\lambda_2$ probes (Figure 1B). The region of this EcoRI insert which hybrides to $(V+C)\lambda_2$ is 5' to that of the $C\lambda_1$ gene; when

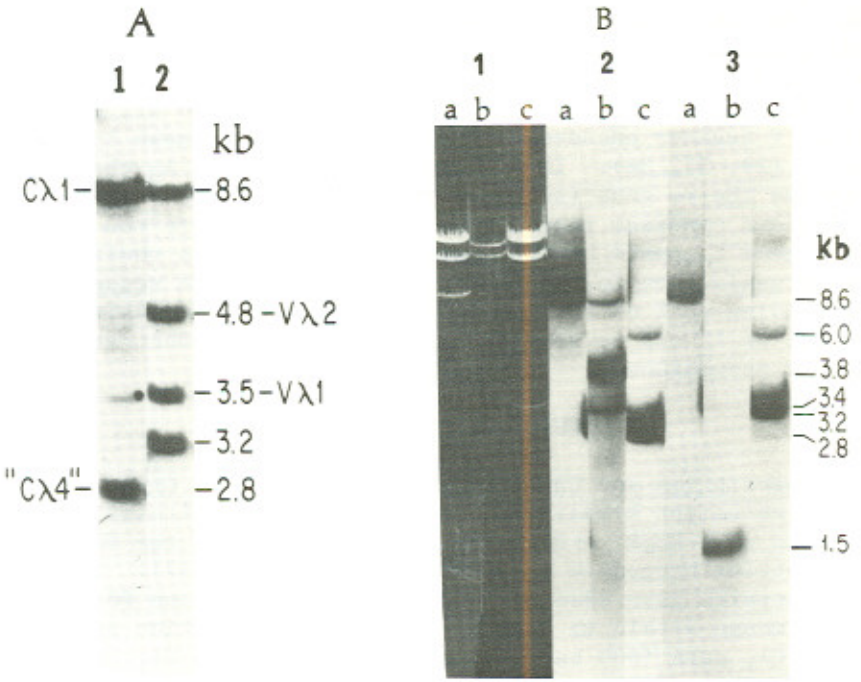

FIGURE 1A. BALB/c embryo DNA digested with EcoRI and hybridized with 1) $C\lambda_1$ or 2) $(V+C)\lambda_2$. Fragment sizes (in kilobases (kb)) were determined by comparison with Hind III-digested λ phage DNA as a size marker.

FIGURE 1B. Cloned DNA fragments containing $C\lambda_1$- or $(V+C)\lambda_2$-hybridizing sequences. Duplicate samples of cloned DNA were electrophoresed in agar, blotted according to Southern and hybridized to either $C\lambda_1$ or $(V+C)\lambda_2$. Section 1: ethidium bromide stain of gel. The top two bands in each sample are phage arms of λWES. Section 2: hybridized to $C\lambda_1$. Section 3: hybridized to $(V+C)\lambda_2$. Lane a: clone Ig25 digested with EcoRI, showing the 8.6 kb fragment hybridizing with both $C\lambda_1$, and $(V+C)\lambda_2$. Lane b: Ig25 digested with KpnI. Lane c: 10A1 digested with EcoRI showing a band at 2.8 kb hybridizing with $C\lambda_1$ and a band at 3.2 kb hybridizing to $(V+C)\lambda_2$. The weak partial band at 6.0 kb in clone 10A1 can be seen to hybridize with both probes.

Ig25 insert DNA is digested with KpnI, the 1.5 kb band, at the 5' side of $C\lambda_1$ (2) hybridizes with $(V+C)\lambda_2$ but not $C\lambda_1$ and the 3.8 and 3.4 kb fragments hybridize only with $C\lambda_1$, as expected. Similarly, clone 10A1, from the 6 kb fragment, when completely digested with EcoRI, revealed two fragments: one at 2.8 kb hybridized only to $C\lambda_1$, and the other at 3.2 kb hybridized only to $(V+C)\lambda_2$. Thus both clones contained two Cλ genes.

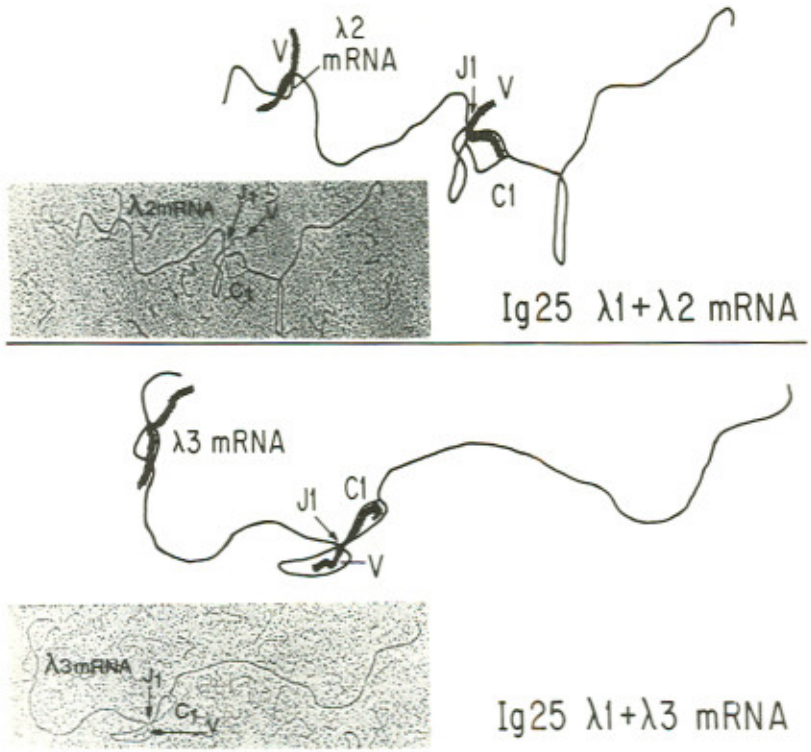

FIGURE 2. Electron micrographs and line drawings of clone Ig25 DNA. R-loops of Ig25 double-stranded insert (8.6 kb) DNA with λ_1 and λ_2 mRNA (top panel) or λ_1 and λ_3 mRNA (bottom panel).

We were able to demonstrate that the clone containing $C\lambda_1$(Ig25) also carried $C\lambda_3$ and that $C\lambda_2$ and $C\lambda_4$ were closely linked (in clone 10A1)(37). Comparisons of the r-loop structures formed by hybridization of DNA from clone Ig25 with a mixture of λ_1 and λ_2 mRNA or λ_1 and λ_3 mRNA showed a larger, more open loop structure with λ_3 mRNA, indicating a greater degree of homology (Figure 2). Similarly, when the 3.2 kb EcoRI insert of clone 10A1 was hybridized with λ_2 or λ_3 mRNA (Figure 3), more homology was seen with λ_2 mRNA. The 2.8 kb band of clone 10A1 was identical to the 2.8 kb band cloned from embryo DNA by restriction enzyme mapping and DNA heteroduplex analysis. Each of the four Cλ gene segments was shown by r-looping to carry its own Jλ segment approximately 1.3 kb to the 5' side (also see J sequence data below).

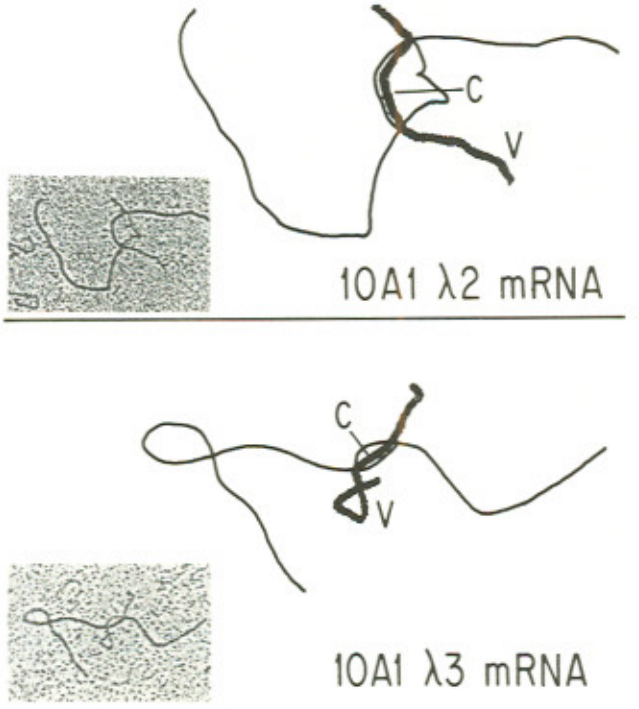

FIGURE 3. Electron micrographs and line drawings of DNA from clone Ig10A1. R-loops of the double-stranded 3.2 kb fragment from EcoRI-digested Ig10A1 DNA with λ_2 mRNA (top panel) or λ_3 mRNA (bottom panel).

The organization of these two Cλ gene clusters is shown in Figure 4. The $J_3C_3J_1C_1$ organization was shown independently by Miller et al. (39). From the similarity in organization of the two clusters and from the cross hybridization between C_2 and C_3, and C_1 and C_4, we proposed that the two clusters arose by duplication and that this duplication unit most likely also included V at the 5' side of the JCJC cluster (37).

Placement of the Two λV Genes. We showed that $V\lambda_1$ has joined to $J\lambda_3$ in the productive rearrangement of a λ_3 producing myeloma. (See Figure 5 A and B: CBPC-49, a λ_3 myeloma shows a rearranged band at 2.8 kb with a $V(J)\lambda_1$ probe. This band was cloned and shown by heteroduplex and r-loop analysis to contain $V\lambda_1$, $J\lambda_3$, and the sequence 3' of $J\lambda_3$ up to the EcoRI

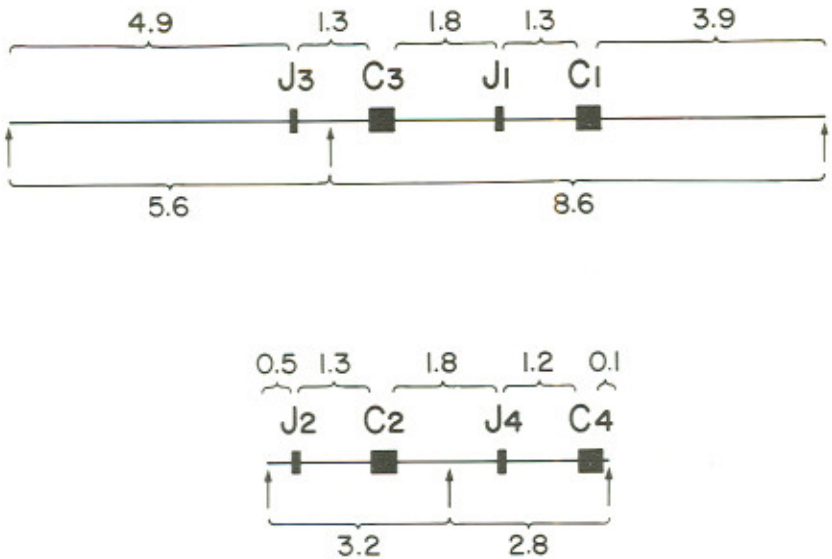

FIGURE 4. Germ line configuration of mouse λ J and C gene segments. Cloned DNA from EcoRI partial digests of BALB/c embryo DNA. The $J_3C_3J_1C_1$ gene segments are from clone Ig S8.2 (not presented) and $J_2C_2J_4C_4$ from clone Ig 10A1 (37). Distances are in kilobases and the J and C regions are designated. Arrows mark the EcoRI cleavage sites. The distance between the two clusters is unknown.

site between $J\lambda_3$ and $C\lambda_3$ (37)). Since V_1 could be shown to be used with C_3 as well as C_1, since V_2 had only been found associated with C_2, and since the V_1 and V_2 gene segments were very homologous (41, 2), we concluded that the probable evolutionary duplication unit was V-JCJC (37). DNA sequence evidence for the J_2-J_3 and J_4-J_1 homology is given below and further supports the above argument.

Further data from which we may deduce the most likely arrangement of the mouse λ locus are given in Figures 5A and 5B. Figure 5A shows a Southern blot of the myeloma Ag8.653 with the $V(J)\lambda_1$ probe where $V\lambda_2$ has undergone two rearrangements (the embryonic $V\lambda_2$ band at 4.8 kb is absent). The two rearranged bands are at 6.5 kb (V_2C_2) and 9.0 kb (9.0 kb is the size predicted for a V_2C_1 rearrangement: the distance from the 5' EcoRI site to the $V\lambda_2$ gene segment (in clone Ig13, ref. 42) is 3.3 kb, the complete V gene is about 0.5 kb, and the distance of $J\lambda_1$ to the 3' EcoRI site (in clone Ig25, ref. 1,

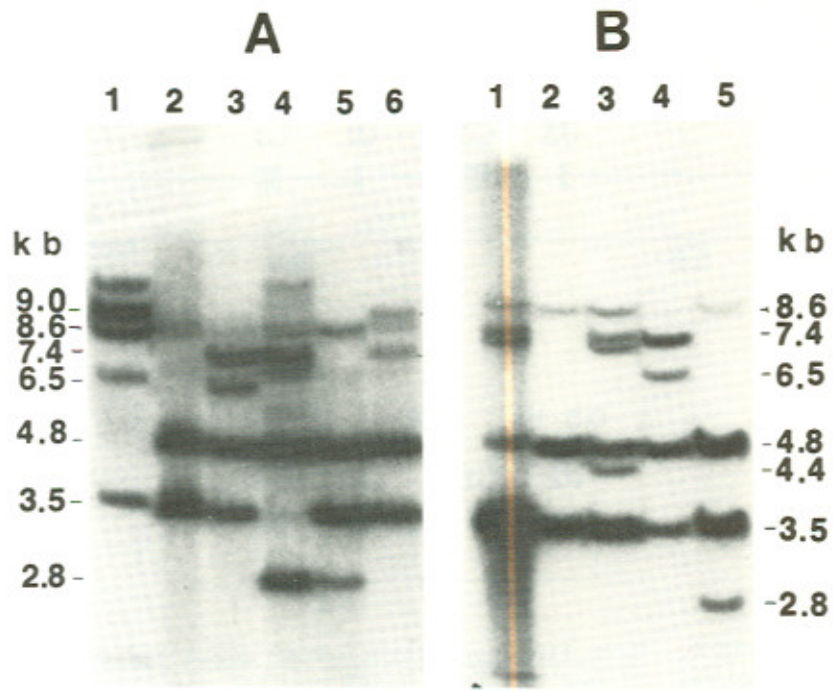

FIGURE 5 A and B. BALB/c embryo, myeloma and hybridoma DNA fragments from EcoRI digestion hybridized to V(J)λ_1 sequences. The probe contains only the V and J regions of λ_1 cDNA. A. DNA from(1) Ag8.653, myeloma fusion parent for hybridoma production,(2) BALB/c embryo,(3) MOPC-315 ($\lambda_2\alpha$), a myeloma producing V_2C_2 light chains (rearranged band at 6.5 kb) and which has also rearranged V_1C_1 (7.4 kb) (See ref. 40 and 37),(4) J558 ($\lambda_1\alpha$) has productively rearranged V_1C_1 and also aberrantly rearranged V_1C_3 (2.8 kb),(5) CBPC-49, a λ_3 producing myeloma (V_1C_3 at 2.8 kb), and(6) P543.6, anti-NP λ-producing hybridoma from T. Imanishi-Kari, possibly V_2C_1 (9.0 kb) as well as V_1C_1 (7.4 kb). B. DNA from(1) SP-2, myeloma fusion parent for 6-2 hybridoma,(2) BALB/c embryo,(3) 6-2, hybridoma (from H.N. Eisen) with 4.4 kb rearranged band, V_2J_3,(4) MOPC-315, and(5) CBPC-49. Fragment sizes are in kb.

see also Figure 4 this paper) is 5.2 kb, producing a rearranged fragment of 9.0 kb). The presence of an embryonic Vλ_1 band in Ag8.653 (in the absence of any Vλ_2 band) rules out the possibility that Vλ_1 lies 3' to Vλ_2 without an intervening JCJC cluster, i.e. the V genes are not clustered (V_2V_1). By a similar argument, the J558 DNA pattern in Figure 5A eliminates

another possibility that $V\lambda_2$ lies 3' to $V\lambda_1$ without an intervening JCJC cluster (the organization is not V_1V_2). In J558 two $V\lambda_1$ gene segments have rearranged, one productively to $J\lambda_1$ (at 7.4 kb) and the other to $J\lambda_3$ (at 2.8 kb); no embryonic $V\lambda_1$ band (at 3.5 kb) can be seen, and yet an embryonic $V\lambda_2$ band (at 4.8 kb) is present. The two cell types, Ag8.653 and J558, are both diploid (as demonstrated by the absence of the embryonic V_2 or V_1 bands, respectively). Therefore, provided that looping out and deletion is the predominant mechanism for V-J joining (16), the two V genes cannot exist without interspersion by a JCJC cluster, i.e. the organization is V-JCJC-V-JCJC.

We suggested above that unusual λ rearrangements may occur (as V_2C_1 in Ag8.653, Figure 5A). This V_2C_1 rearrangement may also have occured in a λ hybridoma (P543.6, from T. Imanishi-Kari, Figure 5A) which appears to produce a protein which shares characteristics of V_2 and C_1 both serologically and biochemically (Reilly, E., B. Blomberg, T. Imanishi-Kari, and H.N. Eisen, in preparation). Another rare λ rearrangement, in the hybridoma 6-2, is shown in Figure 5B. SP-2 is the myeloma which was fused with BALB/c spleen cells for the 6-2 hybridoma production. The rearranged band (the only non-embryo or new band) in 6-2, at 4.4 kb, is consistent only with a rearrangement of $V\lambda_2$ to $J\lambda_3$ (3.3 + 0.5 + 0.6 (the distance of $J\lambda_3$ to the 3' EcoRI site)).[3] Recently, the amino acid sequence of the 6-2 protein was shown to contain the sequence of $V\lambda_2$, $J\lambda_3$, and $C\lambda_3$ (Elliot, B., H.N. Eisen, and L. Steiner, submitted). These rare recombinations (V_2C_1 and V_2C_3 but not V_1C_2) in the approximately 40 λ myelomas and hybridomas studied to date suggest the organization seen in Figure 6.

DNA Sequences of the Four J Regions. Although V_1 is capable of joining with both J_3 and J_1, the ratio of $\lambda_1:\lambda_2:\lambda_3$ is 8:1:1 in the serum and 1.0: 0.7: 0.3 in spleen lymphocytes[3] (35). In order to determine a possible explanation for the lower expression of λ_3 as compared with that of λ_1, to obtain further evidence for an evolutionary duplication unit of JCJC, and to establish whether the J_4C_4 gene segments might be functional, we determined the DNA sequences of the λJ segments of J_2, J_3, and J_4 (Figure 7).

The λ_4 gene is probably a pseudogene. There has been a 2 base pair (bp) deletion within the heptamer recognition sequence (underlined) 5' of J_4 and the donor RNA splice site is missing at the 3' side of J_4. The dinucleotide GT, present at amino acid position 110 in all functional J regions and an obligatory part of all RNA splicing signals (44) is absent in all reading frames of J_4 in this position (43,45). In

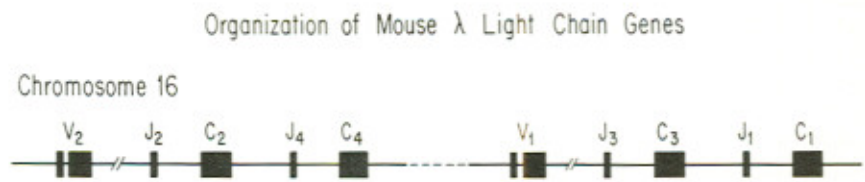

FIGURE 6. Mouse λ light chain locus. The most probable arrangement of the gene segments known to date in the λ light chain locus. Distances between V and its C gene cluster and between each C cluster (shown as -//- and ...) have not been determined.

addition, no protein representative of λ_4 has been described and all serum, hybridoma, and myeloma λ chains can be accounted for by λ_1, λ_2 and λ_3 (20, 29, 34).

The $J_3C_3J_1C_1$ and $J_2C_2J_4C_4$ Sequences Arose by Duplication. The J_1 and J_4 sequences and the J_2 and J_3 sequences are more alike, especially in the noncoding regions (43). The DNA sequences of J_3 and J_2 are 92% homologous in the coding region, 83% (84/101 bp) in the noncoding regions; J_1 and J_4 are 82% homologous in the coding region, 74% (115/155 bp) in the noncoding region. The sequences of J_3 and J_1 are only 77% homologous in the coding region and 30% (49/163 bp) in the noncoding regions; J_2 and J_4 are 75% homologous in the coding region and 35% (50/144 bp) in the noncoding regions. Figure 7 also confirms that each Cλ gene carries its own J sequence to its 5' side: in the case of J_1, J_2, and J_3, the DNA sequences correspond to the previously determined amino acid sequences (20, 38, 34). For J_4, no amino acid sequence is available, but a J-like sequence exists approximately 1.3 kb 5' to the C_4 segment.

Differences Within the Nonamer Sequence 5' of J_3 and J_1 May Account for the Differential Expression of λ_3 and λ_1 Igs. The λ_1 and λ_3 genes share the same $V\lambda_1$ gene and yet the level of expression of λ_3 is much lower than that of λ_1. The ratio of λ_1: λ_2:λ_3 is about 8:1:1 in the serum and 1.0: 0.7: 0.3 in the spleen lymphocytes (35). Consensus nonamer and heptamer sequences 3' of all V gene segments, 5' of all J gene segments and flanking the D gene segments have been proposed as possible recognition sites for a "recombinase" involved in V-J or V-D-J gene assembly (16, 17, 12, 13). These consensus sequences are

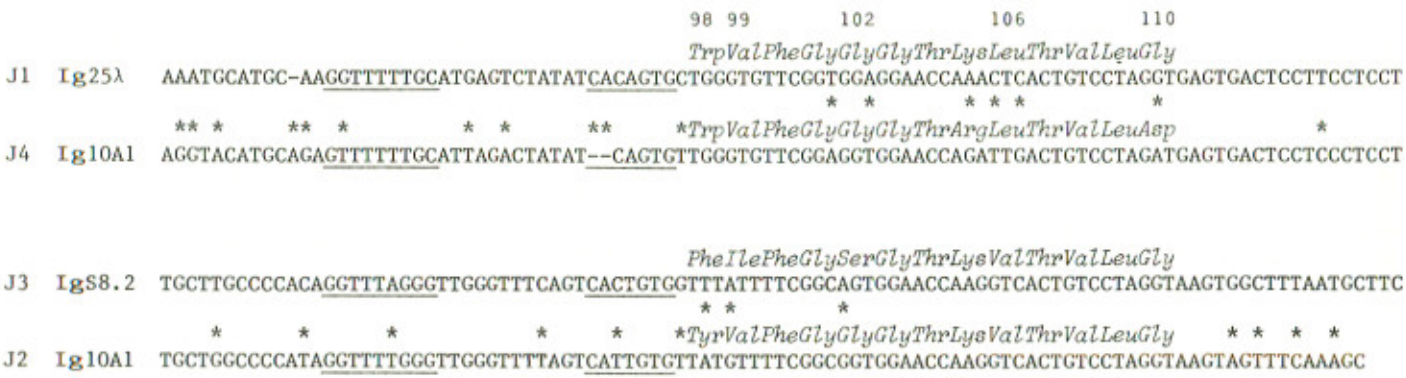

FIGURE 7. Comparison of nucleotide sequences of germ line λ segments and surrounding regions. Extensions of these sequences and sequence strategy are given in ref. 43. *, nonidentical base pairs in comparisons of the J_1-J_4 and J_3-J_2 sequences. Signal nonamer and heptamer sequences 5' to the J regions are underlined. Amino acids encoded by nucleotide sequences are shown in italics. The J_1 sequence was reported previously by Bernard et al. (2).

GGTTTTTGT and CACTGTG. There are two consecutive base pairs changed within the nonamer signal sequence of J_3 as compared with J_1 (Figure 7) (43,45). This difference may cause less efficient joining of V_1 to J_3 and account for a level of expression of λ_3 which is lower than that of λ_1 as seen in serum and in lymphocytes.

CONCLUDING REMARKS

We have described the organization of all of the gene segments known to date for the mouse λ light chain immunoglobulin locus. This locus is unusual and simple in that there are only two V gene segments and four C gene segments, each with its own J segment approximately 1.3 kb to its 5' side. The most likely organization of this locus is shown in Figure 6. These gene segments would account for all the expressed λ light chains, in fact it appears that the λ_4 gene is a pseudogene and not expressed at the protein level. We cloned a crosshybridizing gene segment but it was not further analyzed (37); it is possible that other C gene segments exist in mouse and are not expressed. Recently, wild mice gave 5-7 DNA fragments hybridizing to a $C\lambda_1$ probe, suggesting the presence of additional $C\lambda_1$-like genes (46). Perhaps the weakly hybridizing bands seen in Figure 1A with the $C\lambda_1$ probe are analogous to those seen in the wild mice. The human λ light chain locus has also been found to contain multiple (at least six) copies of closely linked C gene segments (47) but the J gene segments have not yet been located.

The organization of the mouse λ locus (Figure 6) is derived, initially, from the data for physical linkage of $J_3C_3J_1C_1$ and $J_2C_2J_4C_4$ within each cluster (37, 39). The placement of the V gene segments in relation to the two clusters, and the placement of one V-JCJC cluster in relation to the other was deduced from analyses of germ line and rearranged V genes (Figure 5, A and B and ref. 37) and from the fact that V_1-J_1, V_1-J_3 and V_2-J_2 associations occur preferentially in the production of λ_1, λ_2 and λ_3 light chains. The latter preference may simply reflect the proximity of a given V gene segment to its JCJC cluster.

Within a given cluster, eg. V_1-$J_3C_3J_1C_1$, the level of expression of a particular subtype, eg. λ_3 or λ_1, may reflect the efficiency of the V-J joining. The nonamer sequence, 5' of J_3 is different from that of J_1 in two consecutive base pairs (Figure 7). This difference from the consensus sequence may result in less efficient joining of V_1-J_3 as compared with V_1-J_1 and hence a lower level of expression of λ_3 in lymphocytes and serum.

Recently, we cloned the V, J and C gene segments for λ_1 from SJL, a mouse strain with a genetic defect resulting in a low level of λ_1 serum Ig and λ_1-bearing lymphocytes (28). Geckeler et al. (28) proposed that this defect resided in "one of the DNA level recognition sites involved in the translocation event which places the $V\lambda_1$ and $C\lambda_1$ structural genes in a transcriptional unit". This was a reasonable proposal since it appeared that the expression of the λ locus, but not the λ structural locus itself, was affected; the defect behaved as a single gene, and the defect was cis-dominant (normal by λ-low mice gave one-half the normal level of λ_1 serum Ig). We have sequenced the SJL $J\lambda_1$ and $C\lambda_1$ gene segments and surrounding regions. The nonamer and heptamer recognition sequences are identical to those of BALB/c (the SJL nonamer sequence is like that of Mill et al. (45) and different from that of Bernard et al. (2). The sequences for the donor splice site, at the 3' side of J, and the acceptor splice site, at the 5' side of C are intact and identical to those of BALB/c. The poly A addition site is identical to that reported for BALB/c $C\lambda_1$ (40). Within the $C\lambda_1$ coding region there were changes in two bp which give two amino acid differences in the C region. Therefore from the DNA sequence data SJL does not appear defective in the potential for V-J joining or λ_1 expression. The cause of the defect may occur during or after transcription, or perhaps there is cellular suppression of the lymphocytes expressing SJL λ_1 (48).

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