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Proceedings of the National Academy of Sciences of the United States of America, Vol. 78, No. 6, [Part 2: Biological Sciences] (Jun., 1981), 3765-3769.

Proc. Natl. Acad. Sci. USA
Vol. 78, No. 6, pp. 3765–3769, June 1981
Genetics

Organization of four mouse λ light chain immunoglobulin genes

(gene cloning/variable, joining, and constant segments/electron microscopy)

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Contributed by H. N. Eisen, March 10, 1981

ABSTRACT We have cloned four λ light chain constant region (C) genes from mouse embryo DNA. Each carries its own joining (J) segment approximately 1.3 kilobases to its 5′ side. The four C genes occur in two clusters, 5′ $J_3C_3J_1C_1$ 3′ and 5′ $J_2C_2J_4C_4$ 3′, with C_4 being a new Cλ gene. We have also shown that $V\lambda_1$ is joined productively with $C\lambda_3$ in a λ_3-producing myeloma, and it is most likely that $V\lambda_1$ and $V\lambda_2$ are the only Vλ genes. Based on the analysis of the germ line and rearranged variable region (V) λ genes in myelomas we argue that the $V\lambda_1$ and $V\lambda_2$ genes are at the 5′ side of the C_3C_1 and C_2C_4 clusters, respectively. We propose that the two clusters arose by duplication. We also speculate on the role of J-associated DNA sequences in regulation of expression of the λ subtypes.

The immunoglobulin genes occur in three families: one for the heavy chain, and one each for the two light chains, κ and λ. It has been well established that the variable (V) and constant (C) region genes are somatically rearranged during differentiation of lymphocytes (1, 2). In the case of light chains, the V DNA segment, encoding amino acid positions 1–97, joins directly with a joining (J) DNA segment that encodes positions 98–107, thereby creating a complete V gene (3). The J DNA segment is within a few kilobases to the 5′ side (upstream) of the C gene. Production of a complete heavy chain V gene requires joinings of three types of germ-line DNA segments—V, D, and J (4, 5) (the D DNA segments encode primarily the third hypervariable region). Somatic joinings of the two (V, J) or three (V, D, and J) types of germ-line gene segments in various combinations contribute greatly to the amplification of antibody diversity.

The λ light chains, which are present in only about 5% of total serum immunoglobulin in the mouse (6, 7), are of three subtypes, λ_1 (or λ_I) (8, 9), λ_2 (or λ_{II}) (10), and the newly described λ_3 (or λ_{III}) (11). In most mouse strains, λ_1 accounts for about 80–90% of all λ chains (7). Each of the λ subtypes must be encoded by its own constant region (Cλ) gene. Although the molecular structure and organization of the λ_1 V and C genes were the first to be extensively studied (2, 3, 12), the physical relationship among the various λ subtype genes is unknown.

We were interested in determining a molecular basis for the dominant expression of the λ_1 subtype and for the preferential joining of $V\lambda_1$ with $C\lambda_1$ and of $V\lambda_2$ with $C\lambda_2$, as seen in λ myeloma proteins. Additionally, we wanted to know if each Cλ gene carried its own J segment to its 5′ side, or if there were a cluster of J segments as in the κ (13, 14) and heavy chain systems (4, 5).

In this report we describe four Cλ genes and give their molecular organization. We also show that the $V\lambda_1$ DNA segment is joined with the $J\lambda_3C\lambda_3$ DNA segment in the complete λ_3 gene active in a λ_3 myeloma. We argue that the probable order of the various λ gene segments is V_1–$J_3C_3J_1C_1$ and V_2–$J_2C_2J_4C_4$, in which J_4C_4 designates a new Cλ gene.

MATERIALS AND METHODS

Bacteria and Phages. Phage Charon 4A was obtained from F. Blattner (University of Wisconsin, Madison, WI) (15). The λgtWES-λB was from P. Leder (National Institutes of Health) (16). *Escherichia coli* 803 (r_k^-, m_k^-, Su III^+) was originally from K. and N. E. Murray (University of Edinburgh). Lysogens used for preparation of packaging mixtures, BHB 2688 [N205 $recA^-$ (λimm_{434}b2*red3* Eam4 Sam7)/λ] and BHB 2690 [N205 $recA^-$ (λimm_{434} cI_{ts}b2*red3* Dam 15 Sam7/λ] were obtained from B. Hohn (Friedrich Miescher Institute) (17).

Preparation of Mouse Embryo Libraries and Cloned DNA Fragments. *Eco*RI partial libraries in bacteriophage λ were prepared as described (18). Preparation of high molecular weight DNA, preparative agarose gel electrophoresis, and extraction of DNA from agarose have been reported (1). Ligation, transfection, and plaque screening of cloned DNA with nick-translated probes were performed as described (19). All cloning experiments were performed under P3-EK2 conditions in accordance with the National Institutes of Health guidelines for recombinant DNA research, issued in 1976.

Gel Blotting of DNA Fragments. Cellular and cloned DNA fragments produced by restriction enzyme cleavage were transferred from agarose gels to nitrocellulose filters according to the procedure developed by Southern (20).

Electron Microscopy. Procedures for preparing single-stranded and double-stranded DNA·mRNA hybrids, DNA heteroduplexes, and R hybrids have been described (21).

Other Procedures. Purification of λ chain mRNAs of λ_1 (H2020), λ_2 (MOPC315), and λ_3 (CBPC49) has been described (1). The hybridization probes were nick-translated cDNA prepared from MOPC315-containing (V + C)λ_2 (a gift from R. Schwartz and M. Gefter), 600 base pairs complementary to $C\lambda_1$ from an *Hha* I/*Hae* III digest of the B1 plasmid of H2020 cDNA (V + C)λ_1 (19), or 330 base pairs from the *Hha* I/*Hae* III digest of B1-H2020 λ_1 containing only the V and J regions [V(J)λ_1 probe].

RESULTS

Identification of Four *Eco*RI Fragments Carrying Cλ Sequences. High molecular weight total cellular DNA was extracted from kidneys of three mouse strains and digested with *Eco*RI endonuclease, and the resulting DNA fragments were analyzed by the Southern gel blotting technique. The hybridization probe was either (V + C)λ_2 cDNA (Fig. 1*A*) or $C\lambda_1$ cDNA (Fig. 1*B*). With (V + C)λ_2, we usually saw four bands, at 8.6, 4.8, 3.5, and 3.2 kilobases (kb). There was an additional

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Abbreviations: V, variable; C, constant; J, joining; kb, kilobase(s).

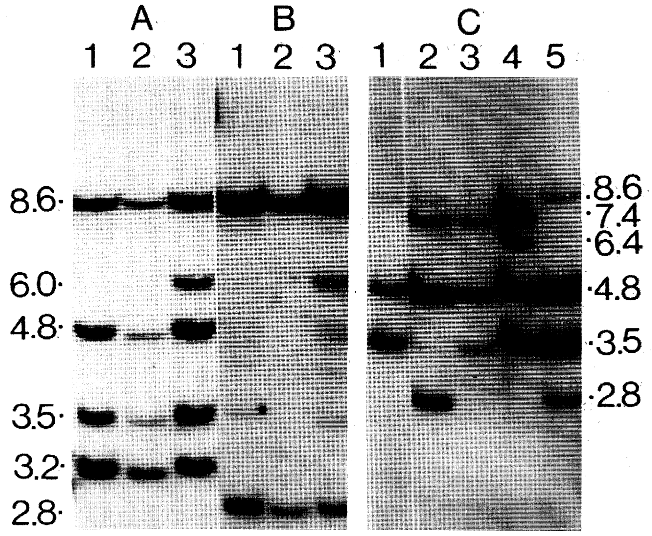

FIG. 1. Embryo-type or myeloma DNA fragments from various mouse strains containing (V + C)λ_2-hybridizing, Cλ_1-hybridizing, or V(J)λ_1-hybridizing sequences. (*A* and *B*) Southern blot of *Eco*RI-digested kidney DNA from BALB/c (lane 1), SJL (lane 2), and C57BL/6 (lane 3) hybridized with probe (V + C)λ_2 (*A*) or Cλ_1 (*B*). (*C*) Embryo and myeloma DNA from BALB/c embryo (lane 1), λ_1 myeloma J558 (lane 2), λ_1 myeloma MOPC104E (lane 3), λ_2 (and abnormal λ_1) myeloma MOPC315 (lane 4), and λ_3 myeloma CBPC49 (lane 5) hybridized with the V(J)λ_1 probe. Fragment sizes (shown in kilobases) were determined by comparison with *Hin*dIII-digested λ phage DNA as size marker.

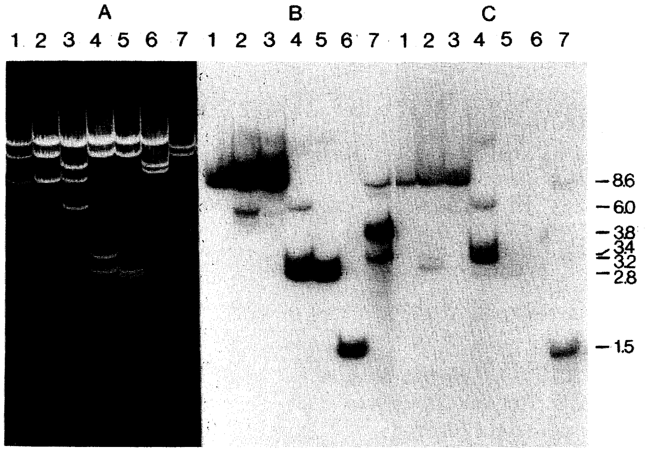

FIG. 2. Cloned DNA fragments containing (V + C)λ_2-hybridizing or Cλ_1-hybridizing sequences. Digests of cloned BALB/c embryo DNA fragments as follows: lanes 1–6, *Eco*RI digest of Ig25λ (lane 1), Ig16.30 (lane 2), IgS6.2 (lane 3), Ig10A1 (lane 4), Ig16.E5 (lane 5), and IgS3 (lane 6); lane 7, *Eco*RI/*Kpn* I digest of Ig25λ. (*A*) Ethidium bromide strain of Southern blot. The top two bands in each sample are phage arms: lanes 3 and 6 are CH4A; the others are λWES. Duplicate samples on the same Southern blot were hybridized with probe Cλ_1 (*B*) or (V + C)λ_2 (*C*).

band at 6.0 kb that usually was faint but in the case of C57BL/6 DNA in this gel it was strong. This band was shown to arise from partial digestion (see next section). The 3.5- and 4.8-kb bands represent Vλ_1 and Vλ_2 genes, respectively, as reported (19). The Vλ_1 gene was detected by its established cross-hybridizability (19) with the V part of the (V + C)λ_2 probe. The amino acid sequence of Cλ_2 and Cλ_3 differs by only five residues between position 120 and the carboxy terminus (11), and we therefore expected to detect the Cλ_2 and Cλ_3 genes with the (V + C)λ_2 probe. On the other hand, Cλ_1 and Cλ_3 proteins differ by approximately 30 amino acids of 94 (11) and we would not expect cross-hybridization. Therefore, the two bands at 8.6 and 3.2 kb seen with the (V + C)λ_2 probe are likely candidates for the Cλ_2 and Cλ_3 genes.

The Cλ_1 probe (Fig. 1*B*) revealed one major band at 8.6 kb, known to contain the Cλ_1 gene (19), and another band at 2.8 kb, seen previously (13) but not characterized. The band at 2.8 kb did not cross-hybridize with the (V + C)λ_2 probe (Fig. 1*A*) and therefore does not contain the Cλ_2 gene and also probably not the Cλ_3 gene. It possibly represents a new Cλ gene which we tentatively refer to as Cλ_4. The partial band at 6 kb as well as the band at 8.6 kb hybridized with both probes and will be further described below.

Clones of the 8.6-, 6.0-, and 2.8-kb DNA Fragments. The 8.6-, 6.0-, and 2.8-kb fragments were enriched by preparative gel electrophoresis and cloned in λWES phage. A Southern blot of the phage DNA of these three types of clones, Ig16.30, Ig10A1, and Ig16.E5, is shown in Fig. 2. Clone Ig16.30, containing an 8.6-kb *Eco*RI fragment and selected by hybridization with (V + C)λ_2, was found to be identical with our previous Cλ_1 clone, Ig25λ (19), based on restriction enzyme digestion and heteroduplex formation (data not shown). Both clones also hybridized with the (V + C)λ_2 and Cλ_1 probes. When Ig25λ DNA was cleaved with both *Eco*RI and *Kpn* I, the 1.5-kb fragment on the 5′ side of this clone (4) hybridized with the (V + C)λ_2 but not the Cλ_1 probe. The 3.4- and 3.8-kb fragments hybridized with Cλ_1, as predicted from previous restriction map analysis (3), but did not hybridize with (V + C)λ_2. Because the Ig25λ clone does not contain V sequences (19), these results suggested that this insert carried either the Cλ_2 or Cλ_3 gene in addition to the Cλ_1 gene (see next section).

Clone Ig10A1, although derived from the 6-kb region of the agarose gel, produced two fragments of 3.2 and 2.8 kb upon complete *Eco*RI digestion (Fig. 2*A*). The 3.2-kb fragment hybridized with (V + C)λ_2 (Fig. 2*C*) but not with the Cλ_1 probe (Fig. 2*B*) or V(J)λ_1 (data not shown), suggesting that this fragment contains either the Cλ_2 or Cλ_3 gene. The other *Eco*RI fragment of this clone, at 2.8 kb, hybridized with the Cλ_1 probe but not with the (V + C)λ_2 probe and is indistinguishable from the 2.8-kb insert of clone 16.E5 containing the Cλ_4 gene. Thus, clone Ig10A1 also carries two Cλ genes: Cλ_2 or Cλ_3 on the 3.2-kb fragment and Cλ_4 on the 2.8-kb fragment. This conclusion is also supported by the fact that the weak band at 6 kb in Ig10A1, representing the partial digestion, hybridized with both the (V + C)λ_2 and Cλ_1 probes. Another distinct clone, IgS3, derived from a library of an *Eco*RI partial digest, contained a 1.5-kb *Eco*RI fragment hybridizing with Cλ_1 but not (V + C)λ_2. This clone has not been characterized further.

Identification of J_3 and C_3 Segments and Their Linkage to J_1 and C_1 Segments. Fig. 3 *A* and *B* shows electron micrographs of hybrids formed between the double-stranded 8.6-kb *Eco*RI insert of Ig25λ and a mixture of λ_1 and λ_2 or λ_1 and λ_3 mRNA, respectively. The position of the Cλ_1 gene in Ig25λ has been previously determined to be 3.9 kb from the 3′ *Eco*RI end (19). The λ_2 plus λ_1 mRNA gave a small R loop 0.6 kb from one end and a larger loop 3.9 kb from the other end. In some molecules the larger loop accompanied a 1.3-kb double-stranded DNA loop. We conclude that the larger loop is due to hybrid formation between λ_1 mRNA and the Cλ_1 gene, based on its position from the *Eco*RI ends. The 1.3-kb loop is due to hybridization of λ_1 mRNA to the J_1 DNA segment (19). The other R loop must be due to hybridization of λ_2 mRNA to the second Cλ gene because, as established (19), λ_1 mRNA does not give an R loop at this position. A mixture of λ_3 and λ_1 mRNA also gave the double R-loop structure similar to the one obtained

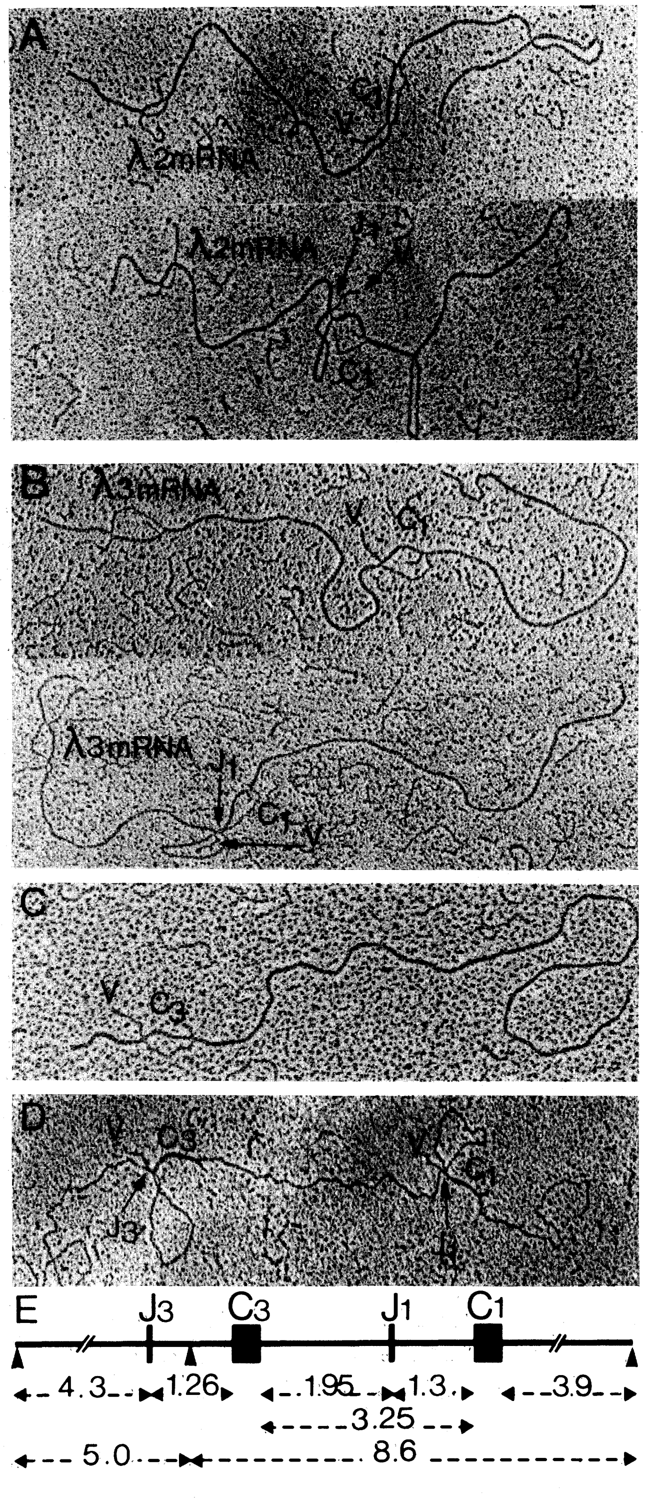

FIG. 3. Electron micrographs of DNA from clones Ig25λ and IgS6.2. R loops of Ig25λ double-stranded insert (8.6 kb) DNA with λ_1 and λ_2 mRNA (*A*), λ_1 and λ_3 mRNA (*B*), or λ_3 mRNA alone (*C*). (*D*) Hybrid of single-stranded IgS6.2 insert (5 + 8.6 + 1.5 kb) DNA with λ_1 and λ_3 mRNA, showing a 1.3-kb DNA loop characteristic of J homology. (*E*) Physical map of clone IgS6.2, which includes the 8.6-kb fragment of clone Ig25λ.

with the mixture of λ_2 and λ_1 mRNAs. However, the R loop at the 5′ side, which is judged to be formed by λ_3 mRNA (Fig. 3*C*), was consistently larger than the R loop formed with λ_2 mRNA. The larger, more open loop structure shown with λ_3 mRNA indicates a greater degree of homology of this mRNA than the λ_2 mRNA with the 5′ Cλ gene. We therefore conclude that the 5′ Cλ gene is Cλ_3 rather than Cλ_2 and that Ig25λ carries both the λ_3 and λ_1 C gene segments.

In order to determine if the Cλ_3 gene also had its own J to its 5′ side, we screened a library of an *Eco*RI partial digest of BALB embryo DNA. IgS6.2 is a representative clone containing a 5-kb fragment to the 5′ side of the 8.6-kb *Eco*RI fragment of clone Ig25 (Fig. 2). Fig. 3*D* shows the RNA·DNA hybrid structure obtained when single-stranded IgS6.2 DNA was hybridized with λ_3 and λ_1 mRNA. The λ_1 mRNA, used as a marker, hybridized to Jλ_1 and Cλ_1 separated by the 1.3-kb intron which appeared as a single-stranded DNA loop. The λ_3 mRNA hybridized with the Cλ_3 gene and also with a short DNA segment located 1.3 kb 5′ to it, giving rise to a 1.3-kb single-stranded DNA loop. We interpret this short DNA segment as Jλ_3. The hybrid structure shown in Fig. 3*D* also indicates that the two Cλ genes are oriented in the same 5′-to-3′ direction because the two mRNAs hybridized with the same DNA strand. Fig. 3*E* summarizes the positions of the various λ gene segments on IgS6.2.

Linkage of Jλ_2Cλ_2 and Jλ_4Cλ_4 Gene Segments. In order to determine that clone Ig10A1 carried the Cλ_2 gene, we made R loops of double-stranded *Eco*RI-digested DNA with either λ_2 (Fig. 4*A*) or λ_3 (Fig. 4*B*) mRNA. The large open loops of the 3.2-kb fragment with λ_2 mRNA compared with the smaller loops with λ_3 mRNA indicated that this gene was indeed Cλ_2, supporting our previous assignment of the Cλ_3 gene to the 8.6-kb fragment. The hybrid formed between the 3.2-kb single-stranded DNA fragment and λ_2 mRNA gave a structure characteristic of the J and C DNA segments, the Jλ_2 segment being 1.4 kb 5′ to the Cλ_3 gene (Fig. 4*C*). Hybridization of λ_2 mRNA to the Jλ_2 DNA segment is also indicated in the left side of Fig. 4*A* by the 1.4-kb double-stranded DNA loop. When λ_1 mRNA was used, only the 2.8-kb fragment gave the similar structure, but at a lower frequency (Fig. 4*D*). A DNA·RNA hybrid of about 400 bp with a 1.3-kb DNA loop was found 0.8 kb from one (5′) end and 0.3 kb from the other (3′) end. Therefore, Cλ_4 also carries its own J.

To determine the relative position and orientation of the Cλ_2 and Cλ_4 genes, λ_1 and λ_2 mRNAs were hybridized individually or as a mixture with clone Ig10A1 DNA partially digested with *Eco*RI. The λ_2 mRNA often gave a hybrid 0.5 kb from one (5′) end of the 6-kb fragment; occasionally the λ_1 mRNA also showed a hybrid 0.3 kb from the opposite (3′) end (Fig. 4*E*). In this micrograph, Jλ_4 is not seen because of weak homology of λ_1 mRNA with the λ_4 gene. The distance between the Cλ_2 and Cλ_4 genes is 3.0 kb, similar to that between Cλ_3 and Cλ_1 (Fig. 3*E*). The positions of the various gene segments on the 6.0-kb insert of Ig10A1 are shown in Fig. 4*F*.

λ_3 Chain Shares Vλ_1 Gene With λ_1 Chain. The CBPC49 λ_3 myeloma (11) DNA gave a characteristic rearranged band at 2.8 kb when analyzed by using the V(J)λ_1 probe (Fig. 1*C*). The size of this rearranged fragment is compatible with joining of Vλ_1 to Jλ_3. The distance from the 5′ *Eco*RI site to the Vλ_1 segment is 1.65 kb (19). Because the complete V gene is about 0.5 kb and the distance from Jλ_3 to the 3′ *Eco*RI site is 0.65 kb (Fig. 3), the expected fragment size of V_1J_3 would be about 2.8 kb (1.65 + 0.5 + 0.65). In contrast, joining of Vλ_2 (22) to Jλ_3 would not explain the 2.8-kb fragment observed in the λ_3 myeloma because it would have produced a 4.5-kb (3.3 + 0.5 + 0.7) fragment.

To demonstrate directly that Vλ_1 is joined with Jλ_3 in the λ_3 myeloma, we cloned and characterized the 2.8-kb fragment (clone Ig284). Electron micrographs of R loops formed between *Eco*RI-digested Ig284 and λ_3 mRNA showed a single loop of about 380 bp with an RNA tail of 350 bp (Fig. 5*A*). An identical

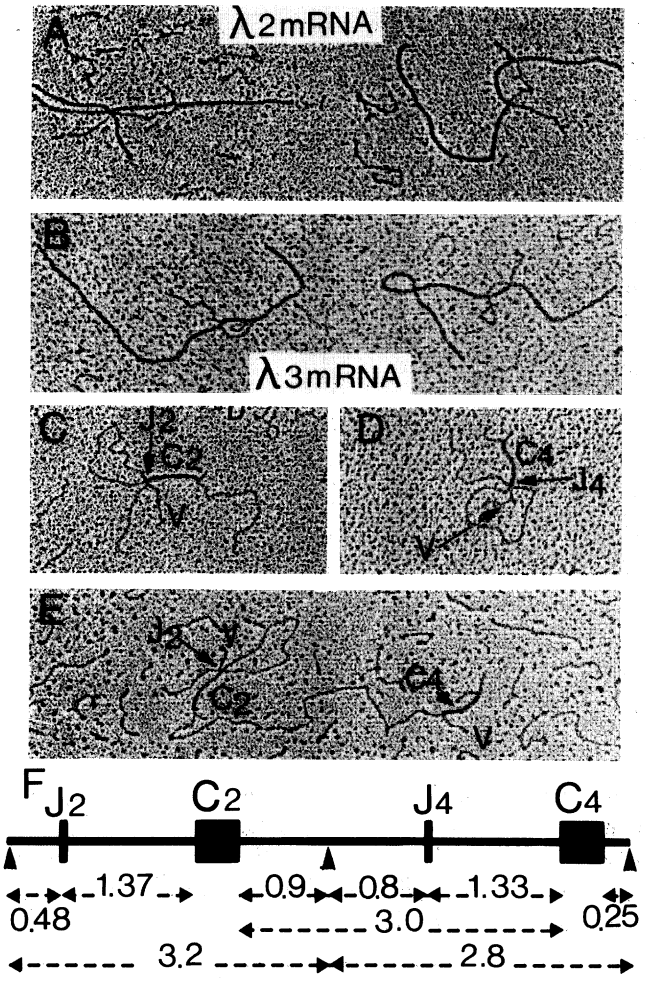

FIG. 4. Electron micrographs of DNA from clone Ig10A1. R loops of the double-stranded 3.2-kb fragment from *Eco*RI-digested Ig10A1 DNA with λ_2 mRNA (*A*) or λ_3 mRNA (*B*). (*C*) Hybrids of single-stranded 3.2-kb fragment with λ_2 mRNA, showing the 1.3-kb DNA loop indicative of a J region. (*D*) Hybrids of the single-stranded 2.8-kb fragment with λ_1 mRNA, showing a 1.3-kb DNA loop. (*E*) Hybrids of the 6.0-kb (3.2 + 2.8) single-stranded fragment from a partial digest with λ_1 and λ_2 mRNA, showing the relative positions of the C_2 and C_4 genes. (*F*) Physical map of clone Ig10A1.

structure was seen with λ_1 mRNA (data not shown). The placement of this V DNA segment 1.7 kb from the 5′ *Eco*RI site is consistent with its being $V\lambda_1$ (19). This idea was confirmed by heteroduplex formation of Ig284 DNA with Ig99 [carrying $V\lambda_1$ and its flanking sequences (19)], which showed that the entire 1.9-kb region extending from the 5′ *Eco*RI site to the V gene is completely homologous (Fig. 5*B*). In order to determine whether the rearranged $V\lambda_1$ gene is connected to $J\lambda_3$, heteroduplex formation of Ig284 with IgS6.2 (Fig. 5*C*), shown above to contain $J\lambda_3$ and $C\lambda_3$, was performed in the presence of λ_3 mRNA. The 0.65-kb duplex was observed immediately 3′ to the $V\lambda_1$ gene which had been marked by hybridization of the premixed λ_3 mRNA. The DNA duplex corresponds to the area bounded by $J\lambda_3$ and its nearest 3′ *Eco*RI site. We conclude that the $V\lambda_1$ segment has joined with $J\lambda_3C\lambda_3$ to produce the λ_3 gene active in the CBPC49 myeloma.

DISCUSSION

We have described four C region genes for mouse λ light chain immunoglobulins, which occur in two clusters, $J_3C_3J_1C_1$ and $J_2C_2J_4C_4$. Only three λ chains have been described to date and therefore we have no information on the function or expression of the $C\lambda_4$ gene. We also do not know if a fifth Cλ hybridizing segment (in clone IgS3) carries a J segment and if it is functional. Because of cross-hybridization between $C\lambda_1$ and $C\lambda_4$ and between $C\lambda_3$ and $C\lambda_2$, we suggest that there was an evolutionary precursor, $C\lambda_I$, for $C\lambda_1$ and $C\lambda_4$ and a precursor $C\lambda_{II}$, for $C\lambda_3$ and $C\lambda_2$ which duplicated in the unit $J_{II}C_{II}J_IC_I$ to give the current gene organization. The conserved 3-kb distance between the $C\lambda_3$ and $C\lambda_1$ genes and between the $C\lambda_2$ and $C\lambda_4$ genes is consistent with this idea.

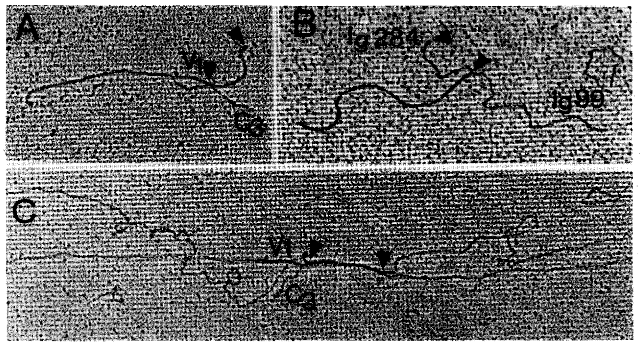

FIG. 5. Electron micrographs of DNA from clone Ig284. (*A*) R loop formed by the 2.8-kb insert and λ_3 mRNA. (*B*) Heteroduplex formed by the inserts of Ig284 and Ig99 ($V\lambda_1$ embryo clone). (*C*) R hybrid formed by the whole phage DNA of Ig284 and IgS6.2 in the presence of λ_3 mRNA. Arrows, distance of 0.65 kb, corresponding to the area between J_3 and its 3′ *Eco*RI site.

We can derive a probable organization of the Vλ genes. There seems to be only two V genes, $V\lambda_1$ and $V\lambda_2$, used for the λ_1, λ_2, and λ_3 subtypes (refs. 19, 23, and 24, and this paper). The λ_2-producing myeloma MOPC315 has two nonembryo (rearranged) bands detected with the $V(J)\lambda_1$ probe (Fig. 1*C*). One band (7.4-kb) is shared with the λ_1-producing myelomas J558 and M104E (Fig. 5) and is most likely the $(V + C)\lambda_1$ rearranged band because it has been shown that MOPC315 also produces a chain in which amino acid position 30 of $V\lambda_1$ is joined directly with $C\lambda_1$ (25). The other band (6.4-kb) is unique for the λ_2-producing myeloma and is thought to be $(V + C)\lambda_2$. The size of this putative $(V + C)\lambda_2$ fragment in M315 is compatible with a joining of $V\lambda_2$ to the $C\lambda_2$ gene: the distance from the 5′ *Eco*RI site to the $V\lambda_2$ gene segment [clone Ig13 (22)] is 3.3 kb, the complete V gene is about 0.5 kb, and the distance of $J\lambda_2$ to the 3′ *Eco*RI site (in clone Ig10A1) is 2.7 kb (Fig. 4*F*), producing a rearranged fragment of about 6.5 kb, as found in MOPC315. Therefore, MOPC315 has rearranged one copy each of $V\lambda_1$ and $V\lambda_2$ and yet maintains both 3.5-kb $V\lambda_1$ and 4.8-kb $V\lambda_2$ embryo DNA fragments (Fig. 1*C*). Because V-J joining almost surely occurs by deletion of the DNA segments between V and J (13, 26), we may rule out an organization of $V\lambda_1$ and $V\lambda_2$ in which there is no intervening Cλ gene. If, for example, the order were V_2V_1 in the germ-line genome, then V_2J_2 joining would have deleted V_1 on one chromosome, and the V_1 rearrangement on the other chromosome would also have removed this V gene from the germ-line configuration; the 3.5-kb $V\lambda_1$ band should then have been absent from MOPC315. The analogous argument applies for the 4.8-kb V_2 band if the order were V_1V_2. Therefore, barring any as yet unknown mechanisms for V rearrangement, such as sister-chromatid exchange, we may tentatively rule out V gene clustering. Because we now know that $V\lambda_1$ may be used with $J\lambda_3C\lambda_3$ [in the CBPC, λ_3-producing myeloma (Fig. 5), and amino acid sequence results of CBPC49 (27)], as well as with $J\lambda_1C\lambda_1$, the most probable gene order for the λ light chains is: V_1–//–$J_3C_3J_1C_1$ and V_2–//–$J_2C_2J_4C_4$. This is without knowledge of the organization of one gene cluster to the other except we know that $V\lambda_1$, $V\lambda_2$, and $C\lambda_1$ all are located on chromosome 16 (28). The proposed organization of V and C genes for λ might explain the fact that, in λ chains, V_1 has been found only with C_1 and C_3 and V_2 with C_2 (11). We would predict that if the C_4 gene is functional, it will use V_2. The V_1 gene may not be efficiently used with C_2 nor, likewise, V_2 with C_1 simply because the distance between these combinations is too great.

Previous data from restriction enzyme mapping of total cellular DNA (19) strongly suggested that there was only one copy per haploid genome of the DNA segment encoding the $V\lambda_1$ and $V\lambda_2$ regions. The absence of the $V\lambda_1$ band (3.5 kb) in J558 (Fig. 6) confirms the point that there is only one $V\lambda_1$ gene per haploid genome. Both copies of the $V\lambda_1$ gene have rearranged in J558: one normally to J_1C_1 (at 7.4 kb) to produce the λ_1 chain, and one nonproductively to J_3C_3 (at 2.8 kb) (unpublished data). This knowledge of the presence of only one $V\lambda_1$ gene, coupled with the amino acid sequence data of λ_1 myeloma proteins showing multiple $V\lambda_1$ sequences (8, 29), again argues that the diversity in myeloma V regions must have been generated somatically.

It has been shown that there is one gene each for $C\lambda_1$ (19) and $C\lambda_3$ and the two C genes share the same V gene, and yet the level of expression of the λ_1 gene is much higher than that of the λ_3 gene. One obvious possible site for regulation of the expression of these two λ subtype genes is the DNA sequence at or around $J\lambda_1$ and $J\lambda_3$. We propose that a "recombinase" for joining of V_1 to J_3 or J_1 either prefers the J_1 region or that mistakes in V–J joining are more often made between V_1 and J_3 than between V_1 and J_1. In fact, the 2.8-kb fragment of the J558 myeloma seems to arise by aberrant V_1J_3 joining (unpublished data). A genetic defect causes a low level of λ_1 chains in the mouse strain SJL. It was previously proposed (30) that this defect may be due to faulty V_1–J_1 joining; because the structural gene products were not affected, it looked like a single gene defect and the effect was *cis*-dominant. Thus, in some cases the unequal expression of λ subtype genes may be directly related to the putative sequence signals at or near the J segments.

We thank M. Courtet, P. Riegert, G. Dastoornikoo, and V. Igras for excellent technical assistance. We also thank R. Schwartz and M. Gefter for the λ_2 cDNA clone, Tommy Meo for initial collaboration, and Richard Maki and Michael Julius for reading the manuscript. The Basel Institute for Immunology was founded and is supported by F. Hoffmann–La Roche and Co., Basel, Switzerland.

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