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Immunology

Physical linkage of the constant region genes for immunoglobulins λ_I and λ_{III}

(gene library cloning/λ light chain gene family)

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ABSTRACT **During differentiation from a stem cell to an antibody-secreting cell, the immunoglobulin genes within a B cell undergo a rearrangement that juxtaposes a variable region gene to a constant region gene. To analyze the genetic organization of an immunoglobulin gene family in nonrearranged, germ-line DNA, we have constructed a recombinant DNA library from randomly cleaved mouse kidney DNA fragments. From this library, we have isolated three overlapping recombinant clones containing the constant region gene for λ_I light chains ($C_{\lambda I}$). These clones spanned 24.9 kilobases of mouse DNA and contained no variable region sequences. Hybridization of these clones with λ_{II} cDNA demonstrated the presence of an additional constant region gene and a joining region 3.2 kilobases 5′ of $C_{\lambda I}$. This gene was tentatively identified as $C_{\lambda III}$ by the absence of an *Ava* I endonuclease site, which is present within $C_{\lambda II}$. The $C_{\lambda III}$ amino acid sequence has recently been reported [Azuma, T., Steiner, L. A. & Eisen, H. N. (1981) *Proc. Natl. Acad. Sci. USA* 78, 569–573] and is very closely related to the $C_{\lambda II}$ amino acid sequence.**

The immunoglobulin λ light chain gene family in the mouse can code for a limited number of polypeptide chains. Only three such chains have been identified: λ_I and its somatic derivatives (1), λ_{II} (2), and λ_{III} (3). λ_I (from myeloma protein MOPC 104E) (4) and λ_{II} (from myeloma protein MOPC 315) (2) differ by 13 amino acids within the variable (V) region and 29 amino acids within the constant (C) region. λ_{III} chains contain a C region very similar to that of λ_{II} (seven amino acid differences) and an unidentified V region (3). These amino acid differences allow cross-hybridization between $V_{\lambda I}$ and $V_{\lambda II}$ (5) and between $C_{\lambda II}$ and $C_{\lambda III}$ (6) and, therefore, the identification of five unique genes: $V_{\lambda I}$, $V_{\lambda II}$, $C_{\lambda I}$, $C_{\lambda II}$, and $C_{\lambda III}$, which may make up the entire λ gene family in the mouse.

The limited genetic complexity of λ genes is quite different from the κ light chain gene family, which apparently contains several hundred V region genes (7) and a single C region gene (8). The genetic disparity between κ and λ genes correlates with the protein ratios found in normal mouse serum, in which 95% of the light chains are κ (9). Presumably, the limited λ gene pool results in a reduced diversity. Therefore, fewer clones of B cells expressing λ chains would be selected, causing a decreased representation within normal serum. Apparently, this does not represent a selective disadvantage for the mouse; the κ genes must be capable of maintaining overall antibody diversity.

To elucidate the organization of λ light chain genes within nonrearranged (germ-line) DNA, we have constructed a complete library of recombinant DNA molecules composing the mouse genome, using the method described by Maniatis *et al.* (10). This technique has been utilized effectively to map the globin gene families in rabbits (11) and humans (12). In this report, we present the linkage of two of the C_λ genes.

MATERIALS AND METHODS

Materials. Restriction endonucleases, *Eco*RI methylase, polynucleotide kinase, bacteriophage T4 DNA ligase, T4 RNA ligase, and DNA polymerase were obtained from New England BioLabs. Synthetic DNA containing the *Eco*RI recognition site was obtained from Collaborative Research (Waltham, MA), nitrocellulose filters from Sartorius, and *S*-adenosyl-L-methionine from Sigma. α-^{32}P-Labeled deoxynucleotides were obtained from New England Nuclear. All cloning strains including bacteriophage λ Charon 4A, the *Escherichia coli* host strains (K802, DP50/*supF*, and CSH18), and the strains for *in vitro* packaging (W3350 and N100) were a gift from F. Blattner.

DNA Preparation. Charon 4A was grown (13) and DNA was extracted (10) as described. After digestion with *Eco*RI restriction endonuclease and annealing of the cohesive ends at 42°C, the outer arms of the phage DNA were prepared by two successive sedimentations through 5–20% linear NaCl gradients (155,000 × *g* for 6 hr at 15°C).

Kidneys were removed from BALB/c mice, cut into several pieces, and rinsed extensively with 0.15 M NaCl/0.01 M Tris·HCl, pH 8.5/0.01 M EDTA to remove excess blood. DNA was prepared as described (14) except that a CsCl density gradient centrifugation replaced pancreatic RNase digestion.

Library Cloning. The mouse germ-line DNA library was constructed as described by Maniatis *et al.* (10) with the following modifications: (*i*) 20-kilobase (kb) DNA was purified on 5–20% NaCl gradients as described above. (*ii*) Blunt end ligation proceeded for 48 hr at 4°C to encourage duplex formation by the eight base pair (bp) linker molecule. (*iii*) T4 RNA ligase was added to the blunt end ligation reaction mixture (15). (*iv*) Excess linker molecules were separated from the 20-kb DNA on a Bio-Gel A-150m column (Bio-Rad). (*v*) Cohesive end ligation was performed with equimolar ratio of vector to insert (13). (*vi*) The recombinant DNA was packaged *in vitro* (16) and amplified in K802. (*vii*) The amplified phage were screened, selected, and purified as described by Walfield *et al.* (17).

Restriction Enzyme Mapping. Isolation of phage miniprep DNA from lysates (16), nick-translations, agarose gels, Southern blots (14), and 3′ end labeling with DNA polymerase (18) were as described. Ambiguities within restriction maps were resolved by two-dimensional restriction enzyme mapping, using low temperature melting (Seaplaque, from Marine Colloids, Rockland, ME) agarose in the first dimension (19, 20).

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Abbreviations: C and V, constant and variable regions of immunoglobulins; *J* region, joining region of immunoglobulin genes; kb, kilobase(s); bp, base pair(s).

Table 1. Cloning efficiency

Efficiency of packaging extract, PFU/μg intact charon 4A DNA	3.0×10^8
Efficiency of recombinant DNA packaging, PFU/μg eukaryotic DNA	2.5×10^6
Total number of independent recombinant phage amplified	1.6×10^6
Probability that any given unique sequence is represented within the library*	99.97%

PFU, plaque-forming units.
* Calculated as described by Clarke and Carbon (25), assuming an average insert length of 15.5 kb and a mouse genome size of 3×10^9 bp.

cDNA Clones. pABλ_I-1 and pλ_{II}-1 are full-length cDNA clones derived from MOPC 104E and MOPC 315 cells, respectively (21). pEJ1-V_λ and pEJ3-C_λ were constructed from pABλ_I-1 by isolating restriction fragments containing only V_λ (230 bp from the 5′ untranslated region to amino acid 57) or C_λ (470 bp, from amino acid 129 to the end of the 3′ untranslated region) sequences from polyacrylamide gels (22) and inserting these fragments into pBR322 (23) by oligo(dG)·oligo(dC) tailing (24).

Electron Microscopy. Heteroduplexes and R-loops with cloned DNA molecules were studied as described (17). Length determinations were made relative to pBR322 linearized with *Hin*dIII.

Recombinant DNA Safety. All procedures for the cloning and propagation of recombinant bacteriophages and plasmids were performed in accordance with the NIH guidelines for research involving recombinant DNA molecules (1978 and 1980) at the P2–EK2 or P1–EK2 level of physical–biological containment.

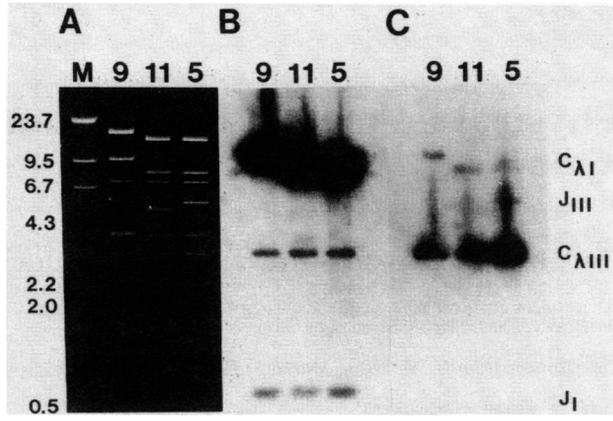

FIG. 1. Hybridization of λ genes in recombinant phage. DNAs from KA9, KA11, and KH5 (abbreviated as 9, 11, and 5) were digested with *Bam*HI and electrophoresed in adjoining lanes on a 1.0% agarose gel. (*A*) Ethidium bromide-stained gel. The DNA was transferred to nitrocellulose, hybridized with pABλ_I-1 (*B*) or with pλ_{II}-1 (*C*), and autoradiographed. The genes contained within each hybridization band are listed at the far right. *J*, joining region. The autoradiograms were overexposed intentionally to highlight faint hybridization bands. The marker lane (M) contains phage λ DNA digested with *Hin*dIII (fragment sizes are given in kb).

RESULTS

Cloning and Isolation of Recombinant Phage. A library of cloned mouse kidney DNA was constructed essentially as described by Maniatis *et al.* (10). Table 1 shows the efficiencies of the cloning protocol used to create a 99.97% "complete" library. From this library 1.2×10^6 amplified phage were screened with nick-translated pABλ_I-1 DNA and three unique

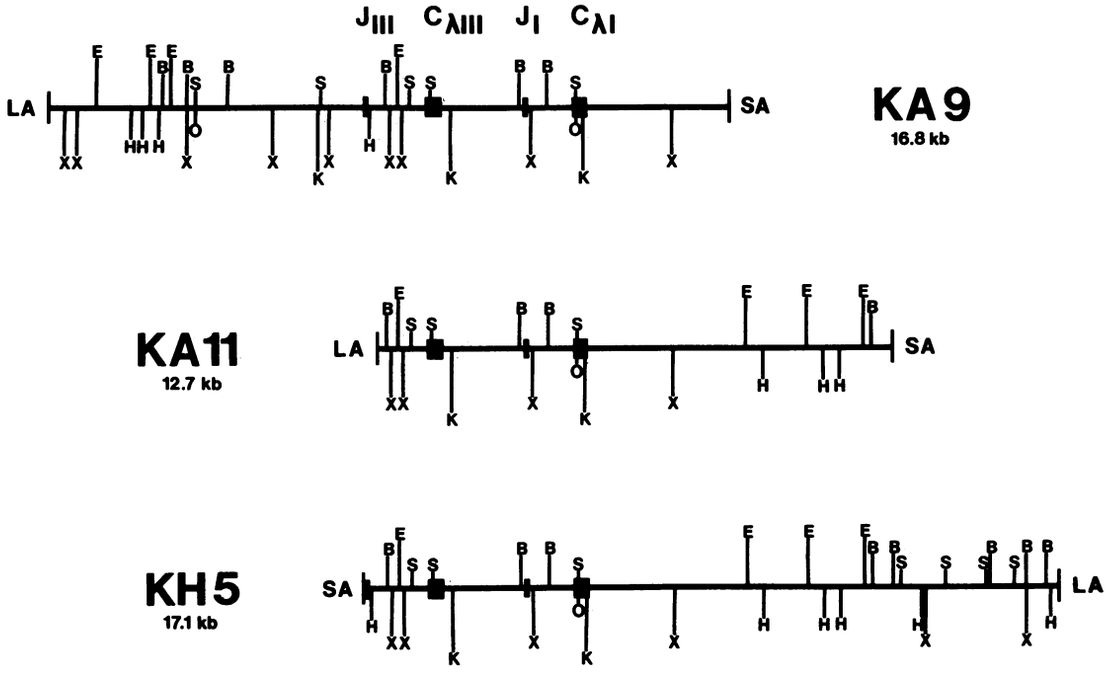

FIG. 2. Restriction enzyme maps of KA9, KA11, and KH5. The individual maps are arranged to align homologous regions of DNA. The bars delineating the ends of the phage inserts indicate *Eco*RI sites, which result from the construction of these phage with *Eco*RI linker molecules. The orientation of the inserts within Charon 4A is designated by SA (short arm of Charon 4A) and LA (long arm of Charon 4A). The black boxes represent the two C_λ genes and their corresponding *J* regions (labeled at top). Enzyme abbreviations are: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; K, *Kpn* I; S, *Sac* I; X, *Xba* I; and O, *Xho* I.

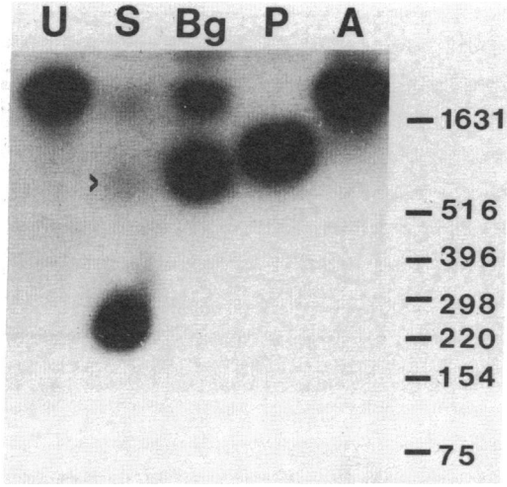

FIG. 3. Autoradiogram of secondary digests of *Xba* I/*Bam*HI fragment. DNA from KA11 was digested with *Xba* I, end labeled with ^{32}P, digested with *Bam*HI, and electrophoresed on a 1% Seaplaque agarose gel. The 2.9-kb *Xba* I/*Bam*HI fragment containing the $C_{\lambda III}$ gene was isolated from the gel and digested with *Ava* I, *Bgl* I, *Pst* I, and *Sac* I in the presence of agarose (19). These reaction mixtures were then electrophoresed on a 4% acrylamide gel (27) and autoradiographed. The molecular weights of the fragments position the first enzyme site 3′ of the *Xba* I site. Thus, the the principal band seen in the *Sac* I digest corresponds to the site 5′ of the $C_{\lambda III}$ gene. The faint band (marked by the arrow), which results from partial digestion by *Sac* I, corresponds to the site within the gene itself (see Fig. 2). The fragment sizes in bp given at the right represent pBR322 DNA digested with *Hin*fI. Enzyme abbreviations are: U, undigested; S, *Sac* I; Bg, *Bgl* I; P, *Pst* I; and A, *Ava* I.

clones were isolated. During plaque purification these phage were shown to contain $C_{\lambda I}$ because they hybridized to pEJ3-$C_{\lambda I}$ DNA and not to pEJ1-$V_{\lambda I}$ DNA. Because $V_{\lambda I}$ and $V_{\lambda II}$ cross-hybridize well (5), none of these phage contain either $V_{\lambda I}$ or $V_{\lambda II}$ sequences. Due to the amplification step the clones (KA9, KA11, and KH5) were purified in multiple copies (3, 2, and 5, respectively). Preliminary restriction enzyme analysis with phage miniprep DNA was used to eliminate redundant clones from further analysis (data not shown).

Restriction Enzyme Mapping. Purified phage DNA was cleaved with seven restriction enzymes (*Bam*HI, *Eco*RI, *Hin*dIII, *Kpn* I, *Sac* I, *Xba* I, and *Xho* I), singly and in pairs, and electrophoresed on agarose gels to determine the molecular weights of the restriction fragments. The DNA was transferred from these gels to nitrocellulose filters (26) and hybridized with nick-translated pABλ_I-1 or pλ_{II}-1 DNA. Fig. 1 is representative of these data and shows that pABλ_I-1 hybridizes strongly to a single *Bam*HI fragment of 9.8 kb in KA9 and 8.0 kb in KA11 and KH5. The molecular weight difference merely reflects that the KA9 insert does not extend to the *Bam*HI site 3′ of $C_{\lambda I}$ (see Fig. 2). Thus, the 9.8-kb fragment in KA9 contains both mouse and phage DNA. A single dark band of 3.2 kb is seen when these clones are hybridized with the pλ_{II}-1 probe (Fig. 1*C*). This 3.2-kb fragment must contain only C region sequences, because the genomic clones do not contain any V region sequences (see above). This gene has been tentatively identified as $C_{\lambda III}$ (see below). In addition to the strong hybridization bands, faint bands are seen that correspond to cross-hybridization between $C_{\lambda I}$ and $C_{\lambda III}$.

The restriction maps of the recombinant phage inserts are shown in Fig. 2. Restriction sites within the DNA represented in more than one clone were verified by parallel electrophoresis of restriction fragments from different phage digested with the same enzyme. Homology regions were also verified by electron microscopy. Heteroduplex analysis of KA9 and KH5 DNAs indicated no mismatching throughout the region of restriction map identity (data not shown). The three clones span a total distance of 24.9 kb of mouse DNA and contain two C_λ genes and their respective *J* regions (see below) (boxed areas in Fig. 2), but no *V* region sequences.

Identification of the $C_{\lambda III}$ Gene. To determine whether the C_λ gene 5′ of $C_{\lambda I}$ is $C_{\lambda II}$ or $C_{\lambda III}$, the 2.9-kb *Xba* I/*Bam*HI fragment containing this gene was digested with *Ava* I, *Bgl* I, *Pst* I, and *Sac* I (Fig. 3). These four enzymes have been shown to cleave within the cDNA sequences for $C_{\lambda II}$ (21). Fig. 3 shows

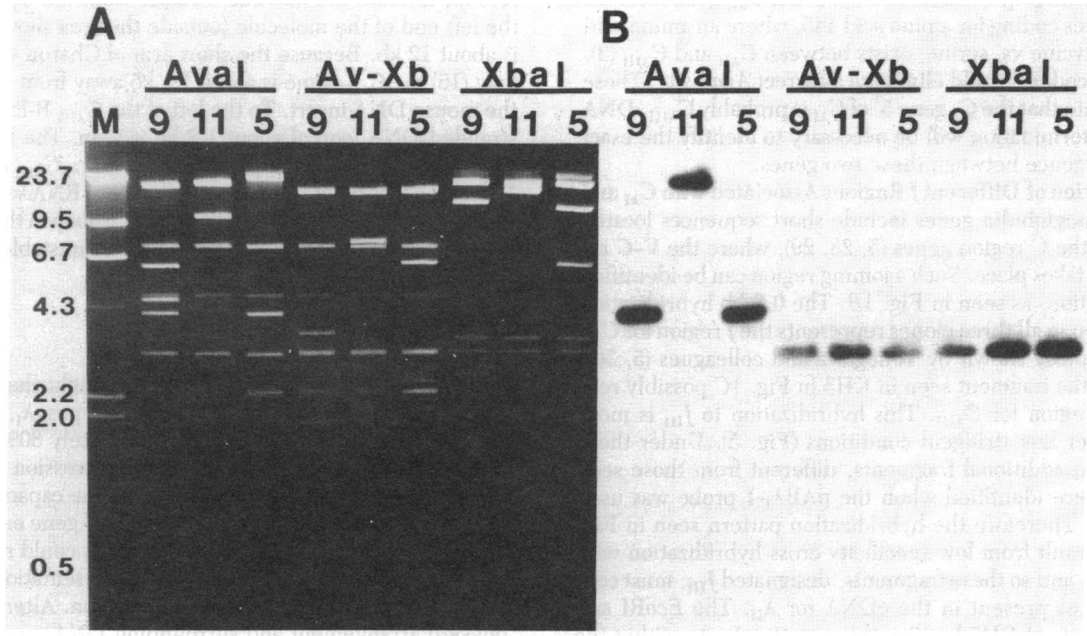

FIG. 4. Agarose gel and Southern blot of KA9, KA11, and KH5 DNA digested with *Ava* I (Av) and *Xba* I (Xb), singly and in combination. DNA was treated as described for Fig. 1. (*A*) Ethidium bromide-stained gel. (*B*) Autoradiogram after hybridization with p$_{\lambda II}$-1. Lanes are identified as in Fig. 1.

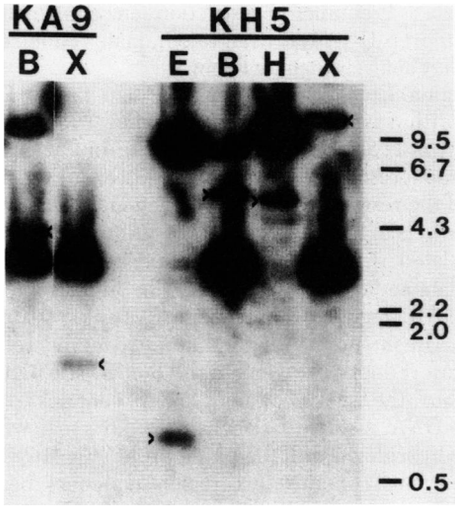

FIG. 5. Demonstration of J_{III} hybridization by Southern blot analysis. DNA from KA9 and KH5 was digested with several restriction enzymes and treated as in Fig. 1. After hybridization with $p\lambda_{II}$-1, the nitrocellulose filter was washed at decreased stringency (45°C in 15 mM NaCl/1.5 mM sodium citrate) (31) to enhance hybridization with *J* region sequences. In all cases, the major hybridization band corresponds to $C_{\lambda III}$. Arrows indicate hybridization bands containing J_{III}. Additional bands seen in the *Bam*HI digests result from cross-hybridization to $C_{\lambda I}$. Note in Fig. 2 that $C_{\lambda I}$ and $C_{\lambda III}$ are located on the same *Eco*RI and *Hin*dIII fragments and on similar molecular weight *Xba* I fragments. Thus, this cross-hybridization is not evident in these digests. The sizes in kb given at the right represent phage λ DNA digested with *Hin*dIII. Enzyme abbreviations are as listed for Fig. 2.

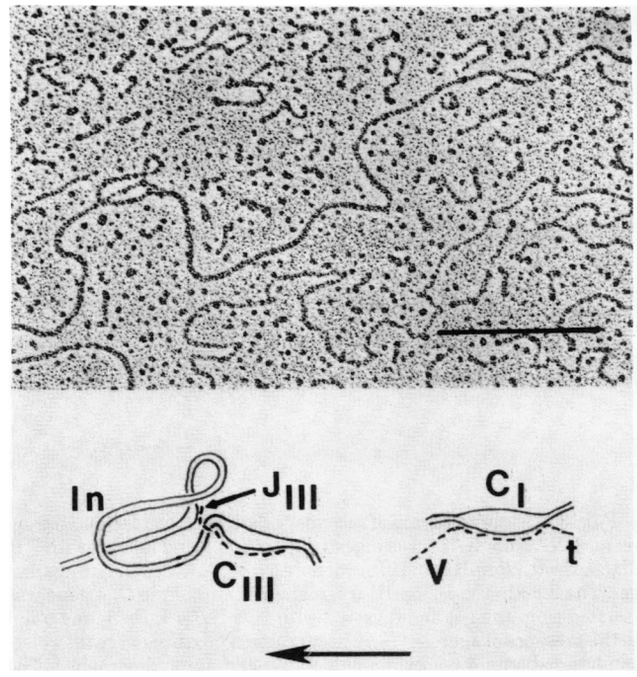

FIG. 6. R-loops with DNA from KH5. The DNA (200 ng) was incubated under R-loop conditions (17) with poly(A)$^+$ RNA (1 μg) prepared from membrane-bound ribosomes of myeloma MOPC-315. In the schematic drawing the arrow points toward the 5′ end of the insert; the dashed track indicates λ_{II} mRNA in the left and λ_I mRNA in the right R-loop. In, intron between $C_{\lambda III}$ and J_{III}. V, *V* region; and t, poly(A) tail of λ_I mRNA. Bar = 1 kb.

that three of the four enzymes (*Bgl* I, *Pst* I, and *Sac* I) cleave within the *Xba* I/*Bam*HI fragment at the sites predicted from the cDNA clone. The fourth enzyme (*Ava* I) does not cleave the *Xba* I/*Bam*HI fragment, indicating the absence of any *Ava* I sites in this C_λ gene. The absence of this site was verified by Southern blots (Fig. 4). In all three genomic clones the 3.2-kb *Xba* I fragment is not cleaved by *Ava* I and a single hybridization band is seen with $p\lambda_{II}$-1. The *Ava* I site in the $C_{\lambda II}$ gene includes the nucleotides coding for amino acid 145, where an amino acid difference (glycine vs. serine) exists between $C_{\lambda II}$ and $C_{\lambda III}$ (3). Some serine codons would eliminate a correct *Ava* I site. These results indicate that the C_λ gene 5′ of $C_{\lambda I}$ is probably $C_{\lambda III}$. DNA sequence determination will be necessary to identify the exact sites of divergence between these two genes.

Identification of Different *J* Regions Associated with $C_{\lambda I}$ and $C_{\lambda III}$. Immunoglobulin genes include short sequences located 1–4 kb 5′ of the C region genes (5, 28, 29), where the *V–C* recombination takes place. Such a joining region can be identified by hybridization, as seen in Fig. 1*B*. The 0.7-kb hybridization fragment seen in all three clones represents the *J* region for $C_{\lambda I}$, as was previously shown by Tonegawa and colleagues (5, 30). By analogy, the fragment seen in KH5 in Fig. 1*C* possibly represents a *J* region for $C_{\lambda III}$. This hybridization to J_{III} is more evident under less stringent conditions (Fig. 5). Under these conditions no additional fragments, different from those seen in Fig. 1, were identified when the $pAB\lambda_I$-1 probe was used (not shown). Therefore the hybridization pattern seen in Fig. 5 does not result from low-specificity cross-hybridization with vector DNA, and so these fragments, designated J_{III}, must contain sequences present in the cDNA for λ_{II}. The *Eco*RI and *Hin*dIII digests of KH5 localize this putative J_{III} to within the 100-bp region between the *Hin*dIII site and the 5′ end of the KH5 insert (see Fig. 2). The intron between $C_{\lambda III}$ and J_{III} is therefore about 1.4 kb.

The presence of the two C_λ genes and of J_{III} on the same cloned DNA is illustrated by R-loop analysis (10 molecules were analyzed) with MOPC-315 RNA (Fig. 6). This myeloma produces both λ_I and λ_{II} mRNAs (21). The R-loop measurements agree with the assignments made by restriction mapping (Fig. 2) within the limits of resolution. The distance between the two R-loops is about 3.6 kb. The distance from the $C_{\lambda III}$ R-loop to the left end of the molecule (outside the area shown in Fig. 6) is about 12 kb. Because the short arm of Charon 4A is 10.9 kb long (16) the $C_{\lambda III}$ gene is about 1.1 kb away from the 5′ end of the mouse DNA insert. To the left of the $C_{\lambda III}$ R-loop a double-stranded DNA loop of about 1.2 kb is seen. The length corresponds roughly to the map distance between $C_{\lambda III}$ and J_{III}. The DNA loop is formed by hybridization of λ_{II} RNA with J_{III}. Such a loop is not seen associated with the $C_{\lambda I}$ R-loop in this molecule, probably because hybridization with *J* is unstable due to the short sequence homology (39 bp).

DISCUSSION

Although λ chains make up only 5% of the light chains in normal mouse serum, there are three defined isotypes: λ_I, λ_{II}, and λ_{III}. λ_I is the predominant isotype; approximately 80% of mouse λ chains are λ_I (32, 33). The differential expression of λ isotypes cannot result from a difference in germline capacity for diversity, because there is apparently a single *V* gene each for λ_I and for λ_{II} (5). Immunoglobulins containing λ_I could react to prevalent antigens, so their increased representation in normal serum could result from antigenic selection. Alternatively, the physical arrangement and surrounding DNA sequences of the λ genes could affect the frequency of V–C joining for the different isotypes and, because expression is dependent on this joining, their relative quantities in mouse serum.

In this report, we demonstrate the physical linkage of $C_{\lambda I}$ and $C_{\lambda III}$ genes within germ-line DNA. We have identified the $C_{\lambda III}$ gene by the lack of a restriction site that is present in the $C_{\lambda II}$ gene. The possibility that this is a pseudogene (34) has not been ruled out. However, this is unlikely because analysis of genomic DNA with several restriction enzymes shows only two C_λ genes hybridizing with a λ_{II} probe (ref. 6; unpublished). $C_{\lambda III}$ is located 3.2 kb 5′ of $C_{\lambda I}$ and, unlike the heavy chain gene cluster (35), contains its own *J* region. We have identified a single *J* region for $C_{\lambda III}$ by Southern blot hybridization and R-loop analysis. It remains to be determined whether there are additional *J* regions located within this area of the DNA. Nucleotide sequence analysis of the area containing the *J* region and the $C_{\lambda III}$ gene itself should allow us to answer this question and to verify the identification of this gene as $C_{\lambda III}$.

The linkage of $C_{\lambda I}$ and $C_{\lambda III}$ raises particular questions concerning the overall gene order of the five λ genes. The deletion model (36) predicts that the *V* gene(s) would be located 5′ of the *C* gene(s). Thus, two alternative gene orders can be presented. First, both V_λ genes could be located 5′ of all three C_λ genes, in the same manner predicted for the heavy chain locus (35), except that each C_λ gene is likely to contain its own *J* region. Second, the *V* genes could alternate between the *C* genes to form separate expression units. These expression units must be located on the same chromosome, because it has been recently shown that all the λ genes are on mouse chromosome 16 (6).

Amino acid sequence analysis of myeloma proteins has demonstrated a strict association of $V_{\lambda I}$ with $C_{\lambda I}$ (37), whereas the *V* gene associated with $C_{\lambda III}$ has not yet been determined. The relative positions of $C_{\lambda I}$ and $C_{\lambda III}$ suggest the usage of a common *V* gene pool. Thus, the differential expression of λ_I and λ_{III} isotypes in normal serum implies predominance of $V_{\lambda I}$ to $C_{\lambda I}$ rearrangements, rather than $V_{\lambda I}$ to $C_{\lambda III}$. Depending on what *V* region is associated with $C_{\lambda III}$, this predominance could be absolute or relative, and it possibly reflects differences within the *J* regions for $C_{\lambda I}$ and $C_{\lambda III}$.

$V_{\lambda I}$ and $V_{\lambda II}$ contain identical nucleotides within the postulated joining sequences located 3′ of all *V* genes (30, 38, 39). If both $V_{\lambda I}$ and $V_{\lambda II}$ genes are located 5′ of all three C_λ genes, an additional specificity, above that proposed from primary sequence analysis, would be required in the control of *V–J* joining within the λ gene family. Further gene mapping within this family and study of the sequences surrounding these genes will provide additional insight into these questions.

Note Added in Proof. DNA sequence analysis has confirmed the presence of a functional *J* region 1.4 kb 5′ of CλIII.

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