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Cloning of mouse immunoglobulin ε gene and its location within the heavy chain gene cluster

(IgE hybridoma/nucleotide sequence determination/deletion of heavy chain genes/gene walking)

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ABSTRACT **Mouse immunoglobulin ε chain gene was cloned from DNA of a hybridoma producing anti-dinitrophenyl IgE, which was constructed by fusing a spleen cell of a BALB/c mouse with a variant clone of MOPC21 myeloma (IgG1 producer). Because a given active heavy chain constant region (C_H) gene is linked to a heavy chain joining segment (J_H) gene at its 5′ side, the expressed C_ε gene of the hybridoma was cloned from a phage library containing partial *Sau*3A digests of IgE hybridoma DNA by using a *J* gene fragment as a probe. Among 6×10^5 phages screened, five positive clones were obtained and three of them were identified as C_ε gene clones by restriction mapping, Southern blot hybridization, R-loop formation, and partial nucleotide sequence determination. The determined nucleotide sequence predicted the amino acid sequence which resembles a part of the C_{H3} domain of human ε chain. The deletion profile of the C_ε gene in various myelomas expressing different C_H genes indicates that the C_ε gene is located between the $C_{\gamma 2a}$ and C_α genes. The linkage (5′-ε-α-3′) was directly confirmed by molecular cloning of the overlapping chromosomal segments from newborn mouse DNA.**

Immunoglobulin heavy chain (*H*) genes consist of a family of variable region (*V*) genes and several constant region (*C*) genes which, in the mouse, are classified into five major classes, μ, γ, α, δ, and ε. In the early phase of B-lymphocyte differentiation, a given *V* sequence is first expressed as a μ chain and, subsequently, the same *V* sequence is associated with other classes of C_H sequences. The phenomenon is called H chain class switch. Based on comparative studies on the rearranged and germ-line *H* genes (1, 2), we have proposed a model that explains H chain class switch by at least two types of recombination events to construct a complete *H* gene. The first type of recombination takes place between a given V_H, a *D*, and a J_H gene segment and forms a complete *V* gene associated with the C_μ gene (3–5). The same *V* gene is subsequently associated with other classes of C_H genes by the second type of recombination which takes place between S regions located in the 5′ side of each C_H gene.

Results supporting this model were also reported from other laboratories (3, 4, 6, 7). Inasmuch as the active C_H gene is expected to contain a *J* gene and a portion of the S region in the 5′ side of the C_μ gene (S_μ) between the *V* and C_H genes according to this model, it is possible to isolate directly any expressed genomic C_H genes, by using the *J* or S_μ DNA segments as probes, from DNAs of myelomas or cell lines that are actively producing particular heavy chains.

Recent studies using cloned DNA segments have revealed basic structure of mouse C_H gene loci (8–11). We have proposed the order of mouse C_H genes as 5′-μ-γ3-γ1-γ2b-γ2a-α-3′ (12), which was subsequently confirmed by studies using Southern blot hybridization experiments in several laboratories including our own (13–17). However, little is known about the genetics of the ε gene, although IgE plays an important biological function as the mediator of allergic reactions (18).

In this paper we report the isolation of mouse ε gene from a library of a mouse IgE hybridoma DNA by using a J_H gene probe. We also report the determination of the C_ε locus between the $C_{\gamma 2a}$ and C_α genes. The linkage between the C_ε and C_α genes was directly confirmed by cloning chromosomal segments that link the two genes. The linkage of 5′-$C_{\gamma 1}$-$C_{\gamma 2b}$-$C_{\gamma 2a}$-C_ε-3′ has been reported (19).

MATERIALS AND METHODS

Materials. Restriction enzymes used are as described (8). Reverse transcriptase from avian myeloblastosis virus was a gift of J. Beard. [α-^{32}P]dCTP (specific activity, 2000–3000 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) and [γ-^{32}P]ATP (>5000 Ci/mmol) were purchased from the Radiochemical Centre (Amersham, England). Myeloma tumor lines used in this study were as described (13). The α cDNA clone (pABα-1) is a generous gift of D. Baltimore (Massachusetts Institute of Technology).

Preparation and Screening of Recombinant Phages. Bacteriophage Charon 28 (20) was used as an EK1 vector and propagated in LE392, a derivative of ED8656 (21). Cloning experiments were carried out in a P3 facility according to the proposed Japanese Guidelines (1978). High molecular weight DNA was purified from the IgE hybridoma or from newborn BALB/c mice as described (13) and was partially digested with *Sau*3A. Partial digests [mean, 15–19 kilobases (kb)] were isolated and ligated with Charon 28 outer fragments, which were produced by *Bam*HI digestion, with T4 ligase as described (22). The recombinant DNA was packaged *in vitro* and phages were screened as described (8, 23). A Charon 4A library containing partial *Hae*III digests of embryonic mouse DNA is a generous gift of P. Leder (National Institutes of Health).

Other Procedures. Southern blot hybridization of digested DNAs was performed as described (8, 24). Cytoplasmic RNA transferred to diazobenzyloxymethyl-paper was hybridized with appropriate probes as described (25). DNA sequencing was performed according to the method of Maxam and Gilbert (26) with slight modifications (8). Electron microscopy of R loops was performed as described (27, 28).

RESULTS AND DISCUSSION

Cloning of the ε Chain Gene. A hybridoma producing anti-dinitrophenyl IgE, IgE-53-569, was constructed by fusing a

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Abbreviations: V and C, variable and constant regions, respectively; *H* gene, heavy chain gene; H chain, heavy chain; kb, kilobase(s).

spleen cell from a BALB/c mouse with P3X63Ag8-6-5-3, a nonsecreting variant clone of mouse plasmacytoma MOPC21 (IgG1 producer) as described (29, 30). The hybridoma was grown subcutaneously. The total cellular DNA was extracted and its partial *Sau*3A digests were ligated to Charon 28 outer fragments to construct a library. About 6×10^5 phages of the library were screened with the ^{32}P-labeled J_{H4} gene segment which was prepared as the 1.5-kb *Hin*dIII/*Eco*RI fragment from the expressed γ1 gene of MC101 myeloma (Igγ1-704) (5, 31). Five positive clones were obtained.

The expressed ε gene in the hybridoma is expected to comprise at least four different segments of DNA—namely, V_H, *D*, *J*, and C_ε gene segments. The hybridoma DNA may contain at least two other forms of the *J* gene; one is the germ-line form of the *J* gene which is linked to the C_μ gene and the other is a part of the unexpressed but rearranged γ1 gene which is derived from the parental plasmacytoma MOPC21. Among three possible forms of J_H genes, only the J_H gene associated with the C_ε gene is expected to be transcribed in the IgE hybridoma. We have tested whether or not the *J*-positive clones contain the C_μ, $C_{\gamma 1}$, and C_ε genes. For this purpose we used, as probes, a 1.3-kb *Hin*dIII fragment of the germ-line μ gene (IgH701) which comprises the C_{H3} and C_{H4} regions of the C_μ gene sequence (10), a 1.8-kb *Hha* I fragment of the γ1 cDNA clone (pcR1-G1-6) (32), and ε chain cDNA complementary to the poly(A)-containing RNA extracted from the IgE hybridoma. None of the *J*-positive clones, however, hybridized with either the C_μ or the $C_{\gamma 1}$ gene probe. Two clones hybridized with the ε chain cDNA. Although the ε chain cDNA is not pure, the expressed J_H genes from the IgE hybridoma are likely to be linked to the C_ε gene. Therefore, they were tentatively identified as ε chain gene clones and named Ch28·M·Igε-1 and Ch28·M·Igε-2, the inserts of which are called Igε-1 and Igε-2, respectively.

Location of *J* Gene. The restriction maps of Igε-1 and Igε-2 were constructed by digestion with various combinations of *Eco*RI, *Bam*HI, *Hin*dIII, and *Xba* I. Igε-1 and Igε-2 are about 15 kb long and their restriction maps are identical except for the absence of the 3′-most *Xba* I cleavage site from Igε-2 DNA (Fig. 1).

To determine the location of the J_H gene sequence, DNA of Ch28·M·Igε-1 was digested with *Eco*RI and *Xba* I and electrophoresed in a 1.0% agarose gel. A Southern blot of the gel was hybridized with nick-translated J_{H4} gene fragment. The *J* gene probe hybridized to an *Eco*RI fragment (2.2 kb) and two *Xba*

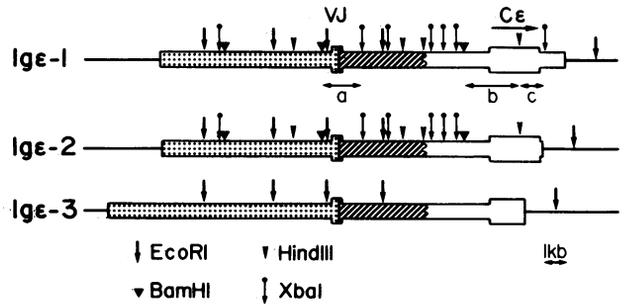

FIG. 1. Restriction maps of Igε-1, Igε-2, and Igε-3. Restriction sites were determined by combined enzyme cleavage. Only *Eco*RI sites were found in Igε-3. Rectangles, insert; solid lines at right and left sides of the inserts, the Charon 28 longer and shorter arms, respectively. The approximate loci of structural gene sequences are represented by wider rectangles. The direction of transcription is from left to right as indicated by a horizontal arrow at the top. The inserts are derived from a chromosomal segment which is composed of V_H (dotted rectangles), J_H with C_μ flanking sequence (hatched rectangles), and C_ε gene (open rectangles) segments.

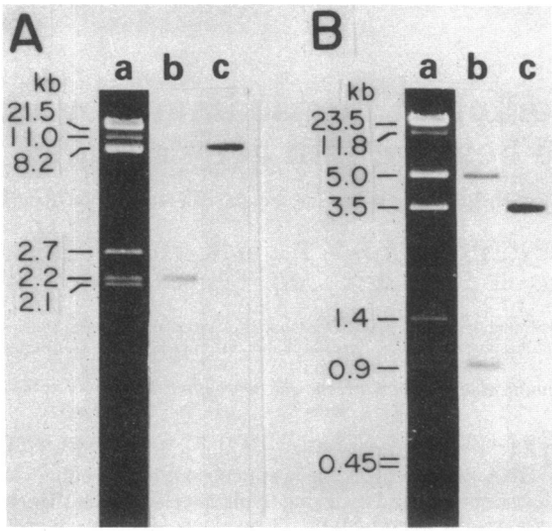

FIG. 2. Southern blot hybridization of restriction fragments of Ch28·M·Igε-1 DNA with ^{32}P-labeled J_{H4} gene fragment and ε chain cDNA. The DNA was digested with *Eco*RI (*A*) or *Xba* I (*B*) and electrophoresed in 1.0% agarose gel. Lanes: a, ethidium bromide stain of restriction fragments; b, Southern blot of the gel hybridized with ^{32}P-labeled 1.5-kb *Hin*dIII/*Eco*RI fragment of Igγ1-704 DNA, which contains the J_{H4} gene and its 3′-flanking sequence; c, hybridized with [^{32}P]DNA complementary to the IgE hybridoma mRNAs.

I fragments (0.9 and 5.0 kb) (Fig. 2, lane b). Ch28·M·Igε-2 DNA gave the identical results. Because the germ-line J_H genes are known to be located on a 6.2-kb *Eco*RI fragment (3), Igε-1 has a DNA rearrangement around the J_H gene, presumably due to *V-D-J* recombination.

The *J* gene fragments of DNAs of the remaining clones were also analyzed. One clone, Ch28·M·Igε-3, produced the 2.2-kb fragment characteristic of DNAs of Igε-1 and Igε-2. *Eco*RI cleavage sites deduced from partial digestion of Ch28·M·Igε-3 DNA agreed well with those of the DNAs of Ch28·M·Igε-1 and Ch28·M·Igε-2 (Fig. 1). Igε-3 extends about 2-kb toward the 5′ side but it is about 1.5 kb shorter at the 3′ side compared with Igε-1.

A fourth clone produced a 2.7-kb fragment of the J_H gene, indicating that this clone has a rearrangement around the J_H gene different from Igε-1, Igε-2, and Igε-3. Although this clone did not hybridize with the $C_{\gamma 1}$ probe, it may be derived from the chromosomal segment of a parental cell line, MOPC21 (a γ1 chain producer), of this hybridoma. The *J* gene may be separated from the $C_{\gamma 1}$ gene by a long stretch of chromosomal segment on the MOPC21 chromosome. Recently, Cory *et al.* (16) reported that the *Eco*RI fragments containing the *J* and $C_{\gamma 1}$ genes are separated by at least one *Eco*RI fragment (7.4 kb) in MOPC21 DNA. A fifth clone was not identified because insufficient DNA was obtained.

Location of the Putative C_ε Gene. Location of the putative C_ε gene was determined by Southern blot hybridization of restriction DNAs with ^{32}P-labeled ε chain DNA complementary to the poly(A)-RNA purified from the IgE hybridoma. When Ch28·M·Igε-1 DNA was digested with *Eco*RI and *Xba* I, it produced 8.2-kb and 3.5-kb fragments, respectively, which hybridized with the ε chain cDNA (Fig. 2, lane c). When Ch28·M·Igε-2 DNA was used, it produced a 7.2-kb *Eco*RI fragment and a 25-kb *Xba* I fragment which hybridized with the C_ε gene probe (data not shown). On the other hand, no significant bands were observed with *Eco*RI digests of Ch28·M·Igε-3 DNA, even after a prolonged exposure of the hybridized filter. Comparison of these results with restriction maps (Fig. 1) in-

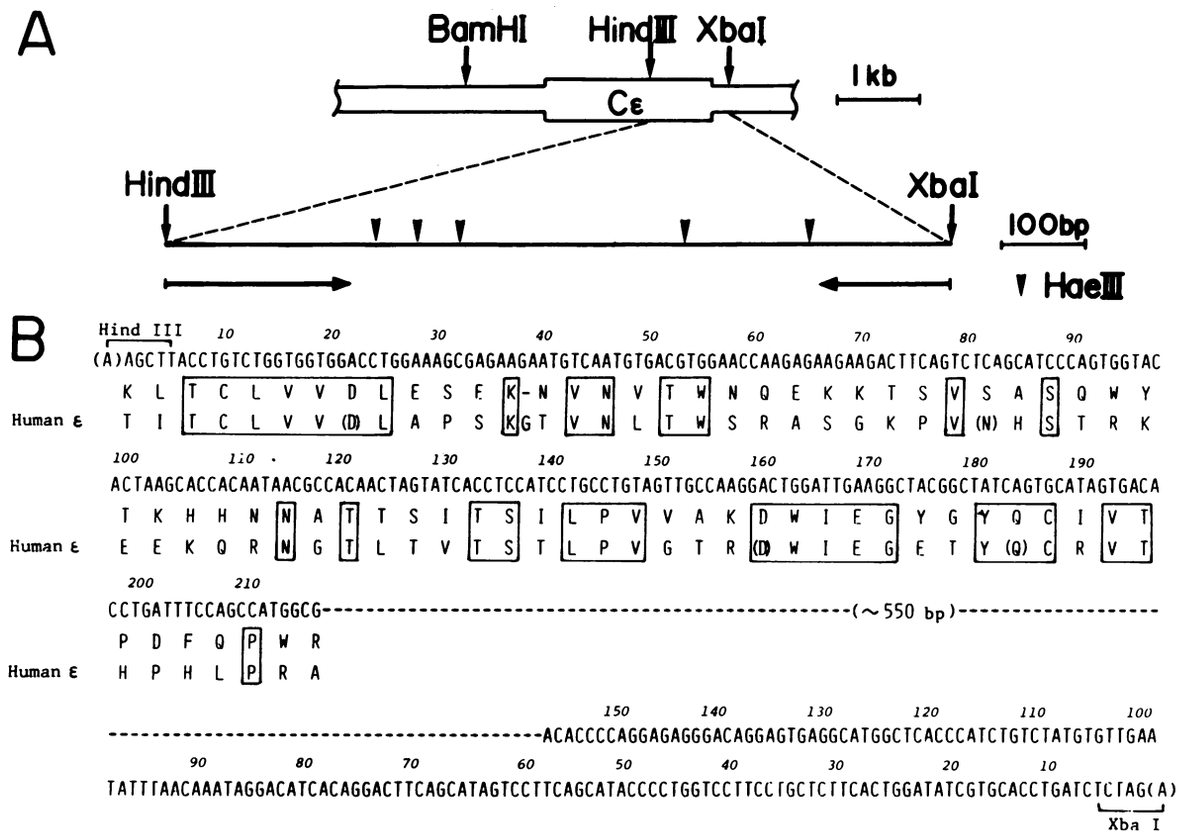

FIG. 3. Partial nucleotide sequences of the C_ε gene. (*A*) Strategy for nucleotide sequence determination. The 0.95-kb *Hin*dIII/*Xba* I fragment (fragment c, Fig. 1) was isolated and its 5′ termini were labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP after bacterial alkaline phosphatase treatment. The 250- and 170-base-pair terminal fragments produced by *Hae* III digestion were subjected to sequence determination (25). Range and direction of sequences read are shown by horizontal arrows. *Hae* III restriction sites were deduced by partial digestion. (*B*) Nucleotide sequence at both termini of fragment c. The nucleotide sequence determined from the *Xba* I cleavage site was translated into the complementary strand. The amino acids predicted by the nucleotide sequence are shown under the coding sequences. The amino acid sequence corresponding to the C_{H3} domain (residues 345–418) of human ε chain (33) is presented at the bottom row and the homologous amino acids are boxed. Amino acids are expressed by one-letter code as follows: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

dicates that the putative C_ε gene is located at the 3′ side of the 3′ *Xba* I fragment (3.5 kb) of Igε-1 DNA.

When the 8.2-kb *Eco*RI fragment of Igε-1 DNA was hybridized with poly(A)-RNA obtained from the IgE hybridoma under the R-loop condition, R loops were formed (data not shown), indiciating that the putative C_ε gene sequence is transcribed in the hybridoma. Analyses of the R loops revealed that the 3′ end of the C_ε gene is located about 2.2 kb 5′ to the *Eco*RI cleavage site in the Charon 28 arm, and the 5′ end of C_ε is about 4.0 kb 5′ to the *Eco*RI site. Such analyses also showed that the C_ε gene is interrupted by at least three intervening sequences.

Partial Nucleotide Sequence Determination. To ascertain that our putative ε gene clones carry the ε chain structural gene sequence, we have determined the partial nucleotide sequence of Igε-1. Because the 3′ terminus of the putative C_ε gene was supposed to be close to the 3′-most *Xba* I cleavage site of Igε-1, the 0.95-kb *Hin*dIII/*Xba* I fragment (fragment c, Fig. 1) was purified and nucleotide sequences of both ends were determined (Fig. 3).

The nucleotide sequence determined from the *Hin*dIII cleavage site codes for 73 amino acids without any interruptions by a termination codon (Fig. 3*B*). The predicted amino acid sequence resembles that of the C_{H3} domain (residues 345–418) of the human ε chain (33). The homology of the amino acid sequence with the human ε chain is 44%; less homology (about 20%) was found with the corresponding portions of mouse μ (34), γ1 (35), γ2a (36), γ2b (9), or α (37) chains. The results unequivocally demonstrate that Igε-1 contains the structural sequence of the C_ε gene and also that the direction of transcription is from left to right. The nucleotide sequence read from the *Xba* I cleavage site in the 5′ direction does not code for amino acid sequences resembling any part of the known heavy chains. The sequence seems to correspond to the 3′ flanking region. The approximate location of the ε gene and the direction of transcription are summarized in Fig. 1.

Comparison of the restriction maps of Igε-1 and germ-line J–C_μ gene region (4, 10) reveals that the segment located 3–6 kb 5′ to the putative 5′ end of the C_ε gene seems to be derived from the J gene and the 5′ flanking region of the C_μ gene. The presence of a V gene sequence was tested by hybridization of DNA fragments with cytoplasmic RNA. mRNA of the IgE hybridoma was fractionated on an agarose gel and transferred to diazobenzyloxymethyl-paper (25). The paper was hybridized with ^{32}P-labeled fragments containing C_ε gene sequence (fragment b in Fig. 1) or the putative V–J gene sequence (fragment a in Fig. 1). Both DNA fragments hybridized to the RNA of 2 kb (data not shown), indicating that the sequence of fragment a is transcribed and present in cytoplasmic RNA. It is likely, but not definite, that fragment a contains the V gene.

Location of the C_ε Gene Within the C_H Gene Cluster. It is well established that deletion of the C_H genes accompanies the H chain class switch (12–17), and the linear arrangement of a

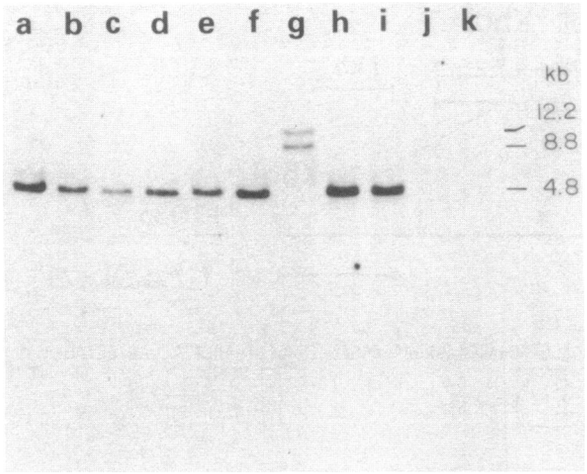

FIG. 4. Hybridization of myeloma DNAs with the C_ε gene probe. DNAs (3 μg) of various myelomas were digested with *Bam*HI and the C_ε gene fragment was detected by Southern blot hybridization with fragment b (Fig. 1) as the C_ε gene probe. Origins of DNA are as follows (the class of heavy chain produced is shown in parentheses): a, liver; b, MOPC104E (μ); c, FLOPC21 (γ3); d, MOPC70A (γ1); e, MC101 (γ1); f, MPC11 (γ2b); g, RPC5 (γ2a); h, UPC10 (γ2a); i, HOPC1 (γ2a); j, MOPC511 (α); k, TEPC15 (α).

given C_H gene can be determined from the deletion profile of the C_H gene in various myelomas expressing different C_H genes. To determine the locus of the C_ε gene within the C_H gene cluster, DNAs of myelomas and livers were digested with restriction enzymes and deletion of the C_ε gene fragments was tested by Southern blot hybridization. A 2.2-kb *Bam*HI/*Hin*dIII fragment (fragment b in Fig. 1) was used as a C_ε gene probe. *Bam*HI digestion of liver DNA produced a 4.8-kb ε gene fragment and the band characteristic to liver DNA was also observed in the DNAs of the myelomas producing μ chain (MOPC104E), γ3 chain (FLOPC21), γ1 chain (MOPC70A and MC101), γ2b chain (MPC11), and γ2a chain (UPC10 and HOPC1) (Fig. 4). On the other hand, DNAs of α chain-producing myelomas (MOPC511 and TEPC15) did not contain any bands that hybridized with the ε gene probe, indicating that the C_ε gene is deleted from both chromosomes of the α chain-producing myelomas. When liver DNA was digested with *Hin*dIII and *Eco*RI, it produced 3.3-kb and 23-kb fragments of the C_ε gene, respectively (data not shown). Similarly, these bands were also found in DNAs of the above myelomas producing μ, γ3, γ1, γ2b, and γ2a chains but not in DNAs of α chain-producing myelomas. These results clearly demonstrate that C_ε gene is located between the $C_{\gamma 2a}$ and C_α genes.

When DNA of RPC5 myeloma (a γ2a chain producer) was digested with *Bam*HI and *Hin*dIII, in each case, two bands (8.8 and 12.2 kb and 4.8 and 5.5 kb, respectively) of the C_ε gene were produced instead of the band characteristic of liver DNA. We have recently cloned an *Eco*RI fragment which contains both the $C_{\gamma 2a}$ and C_ε genes from RPC5 myeloma DNA (19). Characterization of this clone indicates that an 11.5-kb segment including a major portion of the C_ε gene and its 3′ flanking sequence is deleted from the $C_{\gamma 2a}$ gene clone. Appearance of two bands may suggest that both of the homologous chromosomes have deletions at different positions. The significance of such a rearrangement is not clear.

The C_ε Gene Is Located 12 kb 5′ to the C_α Gene. To determine directly the location of C_ε gene, we have isolated a number of clones from germ-line mouse DNA libraries by using the C_ε and C_α gene probes. When we screened a Charon 4A library containing partial *Hae* III digests of embryonic mouse DNA with the α cDNA clone (fragment e, Fig. 5), we isolated a clone called Ch4A·M·Igα-8. The clone was characterized by restriction mapping and Southern blot hybridization and was found to contain the C_α gene (Fig. 5). Then, the 4-kb *Hap* II/*Sac* I fragment of Igα-8 (fragment d, Fig. 5) was used as a probe to screen a Charon 28 library containing partial *Sau* 3A digests of newborn mouse DNA. We obtained three clones, designated Ch28·M·Igα-3, Ch28·M·Igα-13, and Ch28·M·Igα-27. When the same library was also screened with the C_ε gene probe (fragment b, Fig. 5), two clones, Ch28·M·Igε-6 and Ch28·M·Igε-7, were isolated. These clones were characterized by restriction mapping and Southern blot hybridization (Fig. 5). Comparison of

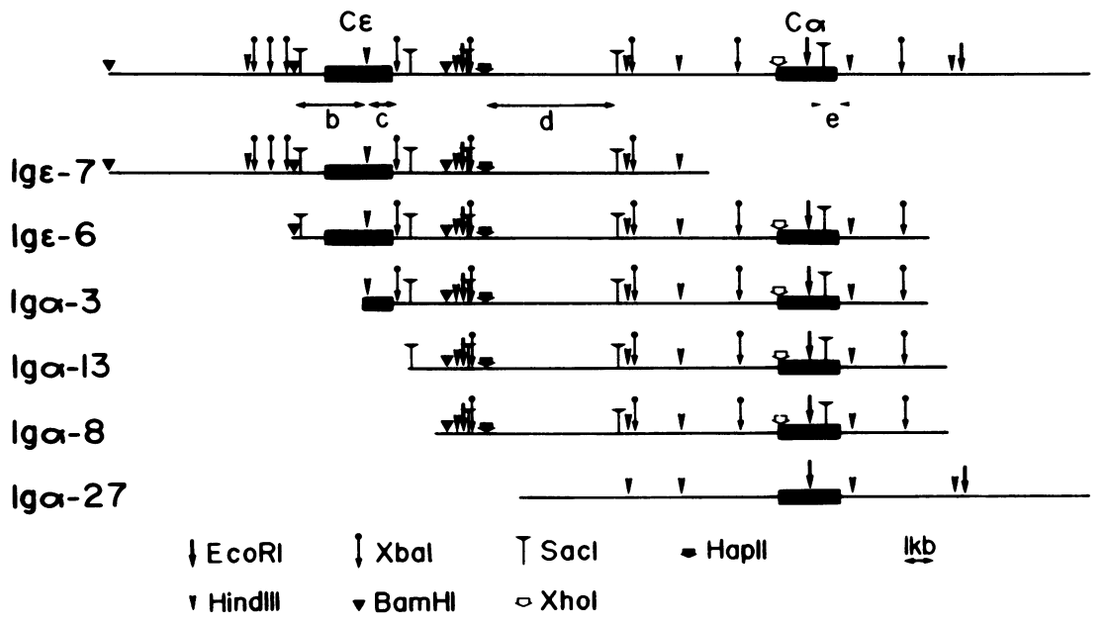

FIG. 5. Restriction endonuclease maps of overlapping cloned fragments between the C_ε and C_α genes. At the top, the chromosomal segment containing C_ε and C_α genes is shown schematically. Structural genes are shown as closed boxes with direction of transcription from left to right. Horizontal arrows under the top line indicate fragments used as probes for screening or Southern blot hybridization. Each clone is shown by a horizontal line with restriction sites. Only *Eco*RI and *Hin*dIII cleavage sites were determined in Igα-27.

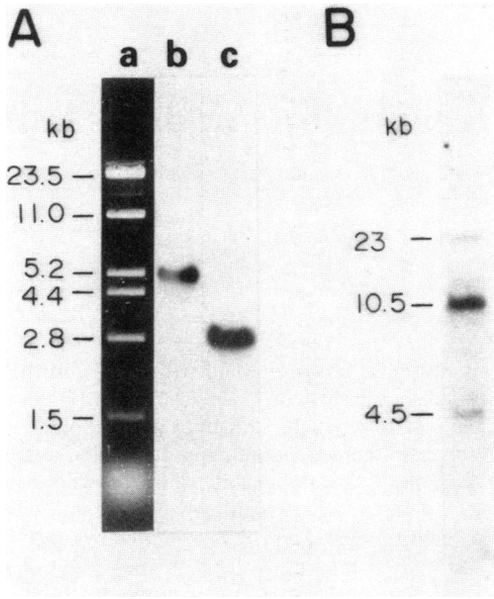

FIG. 6. Southern blot hybridization of Ch28·M·Igα-3 and newborn mouse DNAs with the α and ε gene probes. (*A*) Ch28·M·Igα-3 DNA was digested with *Hin*dIII and electrophoresed in a 1.0% agarose gel. The Southern blot of the gel was hybridized with ^{32}P-labeled fragment e (lane b) or fragment c (lane c). Ethidium bromide stain of the gel is shown in lane a. (*B*) Newborn mouse DNA (3 μg) was digested with *Eco*RI. A Southern blot of the *Eco*RI digests was hybridized with ^{32}P-labeled Ch28·M·Igα-3.

the restriction maps clearly shows that all these clones have some overlaps with each other and that the 3′ portion of the C_ε gene clones and the 5′ portion of the C_α gene clones share the common restriction sites. Most convincingly, Igα-3 contains both the 5.2-kb *Hin*dIII fragment that hybridized with the C_α gene probe (fragment e) and the 2.8-kb *Hin*dIII fragment that hybridized with the C_ε gene probe (fragment c) (Fig. 6*A*). These results clearly demonstrate that the C_ε gene is located at 5′ to the C_α gene and the two genes are about 12 kb apart. It is worth noting that the directions of transcription of the C_ε and C_α genes are identical (6).

Davis *et al.* (6) reported the presence of an 8.5-kb *Eco*RI fragment immediately 5′ to the 10.5-kb C_α gene fragment, which disagrees with our corresponding fragment of 23 kb. We have tested whether or not the 8.5-kb *Eco*RI fragment is present between the C_ε and C_α genes by Southern blot hybridization of newborn mouse DNA. When the total phage DNA of Ch28·M·Igα-3 was used as a probe, *Eco*RI digests of newborn mouse DNA produced 4.6-, 10.5-, and 23-kb bands (Fig. 6*B*). The first two bands coincide with the *Eco*RI fragments deduced from the cloned DNAs, and the last band agrees with our previous observation that both $C_{\gamma 2a}$ and C_ε genes are present on a single *Eco*RI 23-kb fragment (14, 19). Although the reason for the discrepancy is not certain, we conclude that the distance between the C_ε and C_α genes in the germ-line chromosome is 12 kb.

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