Volume 8 Number 24 1980 Nucleic Acids Research

Cloned embryonic DNA sequences flanking the mouse immunoglobulin $C_{\gamma 3}$ and $C_{\gamma 1}$ genes

Jerry M.Adams, Elizabeth Webb, Steven Gerondakis and Suzanne Cory

The Walter and Eliza Hall Institute of Medical Research, Post Office Royal Melbourne Hospital, Victoria 3050, Australia

Received 28 October 1980

ABSTRACT

To investigate the DNA surrounding genes for immunoglobulin heavy chain constant (C_H) regions, we have isolated two clones bearing a Cγ3 gene and two bearing a Cγ1 gene from a library of mouse embryo DNA fragments. The Cγ3 clones span 8.6 kilobase pairs (kb) on the 5' side of the gene and 6.7 kb on its 3' side, while the Cγ1 clones together span 13 kb of 5' flanking sequence and 2.5 kb of 3' flanking sequence. Restriction mapping of the Cγ3 gene indicates that intervening sequences divide the gene into segments of domain size, as in other C_H genes. Hybridization of clone fragments to restriction digests of mouse DNA indicates that both the Cγ1 and Cγ3 genes probably occur as single copies in the genome. Moreover, the entire cloned sequences on the 5' side of both genes appear to be unique in the genome, indicating that no large common sequences flank C_H genes. Restriction data suggest that the Cγ3 gene is 37-40 kb 5' to the Cγ1 gene.

INTRODUCTION

The immunoglobulin heavy chain genes are of considerable interest because this locus undergoes a remarkable series of somatic DNA rearrangements (see (1) for a review). As in light chains, the variable (V) and constant (C) portions of each chain are encoded separately, but each of the C_H genes (μ, δ, γ_1, γ_{2a}, γ_{2b}, γ_3, ε and α in the mouse) shares the same large cluster of V_H genes. Somatic recombination links a V_H gene and one of four joining region (J_H) genes near the C_μ gene (2-5) and the active V_H-J_H gene can subsequently switch from C_μ to another C_H gene by recombination between the 5' flanking sequences of the two C_H genes (5-7). Both V_H-J_H joining (3,8) and switch recombination (9-13) involve deletion of the DNA between the recombining sequences.

Understanding heavy chain expression requires a detailed picture of C_H gene organization. Each C_H gene is thought to occur as a single copy per haploid genome but this has been well documented only for the C_μ gene (8,11). Deletions found in different plasmacytomas suggest that

© IRL Press Limited, 1 Falconberg Court, London W1V 5FG, U.K.

the probable C_H gene order is 5' μ-γ_3-γ_1-γ_{2b}-γ_{2a}-α 3' (8-13), and the linkages C_μ-C_δ (14), $C_{\gamma 1}$-$C_{\gamma 2b}$ (15) and $C_{\gamma 2b}$-$C_{\gamma 2a}$ (16) have been established recently with cloned sequences. An intriguing feature of C_H gene structure has emerged from the finding that cloned $C_{\gamma 1}$ (17,18), $C_{\gamma 2b}$ (19,20), C_α (21), and C_μ (22-24) genes are all divided by intervening sequences into segments which correspond closely to the globular domains of the polypeptides.

To analyze the nature of sequences encompassing C_H genes, we have searched a library of cloned embryo DNA fragments (25) for C_H genes and describe here clones bearing the $C_{\gamma 3}$ and $C_{\gamma 1}$ genes. The $C_{\gamma 3}$ gene is of particular interest because little has yet been published on the structure of the γ_3 chain of mice. We show that the $C_{\gamma 3}$ gene has a domain-like structure very similar to that of the $C_{\gamma 1}$ and $C_{\gamma 2b}$ genes. By hybridizing fragments of the cloned sequences to mouse DNA, using the Southern technique (26), we have determined that the $C_{\gamma 1}$ and $C_{\gamma 3}$ genes occur as single copies in the mouse genome and that no large common sequences occur 5' to C_H genes. The Southern analysis also provides evidence for linkage between the $C_{\gamma 3}$ and $C_{\gamma 1}$ genes. Correlating this data with published work on other C_H clones indicates that the C_H locus is much larger than might have been expected, in excess of 150 kb long.

EXPERIMENTAL PROCEDURES

Phage screening. Phage plaques from a clone library (25) were screened (27) using as probes the cDNA inserts from plasmids pM21γ1.1, pH1γ2a.8 and pY5606γ3.15 (28), labelled by nick translation (29). To reduce background hybridization, filters were preincubated at 65°C in 0.2% Ficoll, 0.2% polyvinyl pyrollidone, 0.2% bovine serum albumin, 0.03 M sodium citrate, 0.3 M NaCl, 5 mM EDTA and 0.1% SDS, then treated at 42° in 50% formamide, 0.02% Ficoll, 0.02% polyvinyl pyrollidone, 0.02% bovine serum albumin, 0.075 M sodium citrate, 0.75 M NaCl, 5 mM EDTA, salmon sperm DNA (0.1 mg/ml) and poly C (40 µg/ml). Hybridization was carried out in the same solution at about 5×10^5 cpm/ml.

Southern analysis. DNA fragments fractionated by electrophoresis on horizontal slab gels of 0.7% agarose were blotted onto nitrocellulose filters (26). Filters were pretreated, hybridized and washed as described (8,30), except that poly C replaced poly A and 5 mM EDTA was included.

Detailed restriction mapping of the $C_{\gamma 3}$ gene. Plasmid DNA from a $C_{\gamma 3}$-

gene bearing Bam HI/Hind III subclone of genomic clone G3.1 was digested with Bam HI and labelled at 3' termini using E. coli DNA polymerase I and (α^{32}P) dATP. After digestion with Hpa I, the 1.7 kb labelled fragment (A in Fig. 3) was purified by sucrose gradient centrifugation. In addition, a purified 3 kb gene-containing Hha I fragment from the same subclone was cleaved with Taq 1 and labelled similarly with (α^{32}P) dCTP. After recutting with Pvu II, the 2 kb labelled fragment (B in Fig. 3) was purified on a 5% polyacrylamide gel. These two fragments were then analyzed by partial digestion with restriction enzymes (31).

RESULTS

Clones bearing large sequences flanking the $C_{\gamma 3}$ and $C_{\gamma 1}$ genes.

From a library of cloned 16-20 kb fragments of mouse embryo DNA (25) which was generated by partial digestion with the Eco RI* activity of Eco RI endonuclease, we have isolated two clones, G1.1 and G1.2, bearing the $C_{\gamma 1}$ gene and two, G3.1 and G3.2, bearing the $C_{\gamma 3}$ gene. G1.2 and G3.1 were found by direct screening (27) of about 1.6 x 10^6 clones from the library, using cloned C_γ cDNA probes (28), while the other two were isolated by screening a portion of the library enriched by R-looping (32).

Since different C_γ nucleotide sequences cross-hybridize to varying extents (33), it was necessary to establish which C_γ gene(s) the clones bore. We hybridized $C_{\gamma 3}$, $C_{\gamma 1}$ and $C_{\gamma 2a}$ probes to replicas of a filter bearing restriction fragments of the genomic clones and, as a control, γ_3, γ_1, and γ_{2a} cDNA plasmids (Fig. 1). In accord with the assignments made, the γ_3 probe hybridized far more strongly to G3.1 (lane 2) and G3.2 (not shown) than to G1.1 (lane 1) or G1.2 (lane 3), while the γ_1 probe hybridized almost exclusively to G1.1 and G1.2. Moreover, the γ_{2a} probe hybridized weakly to all the genomic clones, compared to its hybridization to a γ_{2a} sequence (lane 6), as would be expected from solution hybridization results (33).

We verified the identity of the clones by showing that gene-bearing fragments from each clone hybridized in Southern experiments to the restriction fragments of mouse DNA revealed by authenticated (28) $C_{\gamma 1}$ and $C_{\gamma 3}$ cDNA probes. Thus Fig. 2A shows that in five different digests of embryo DNA, the fragment detected using a gene-bearing probe from G1.1 was the same size as that revealed by the $C_{\gamma 1}$ cDNA probe. Similarly, Fig. 2B shows that a gene-bearing probe from G3.1 recognized the same

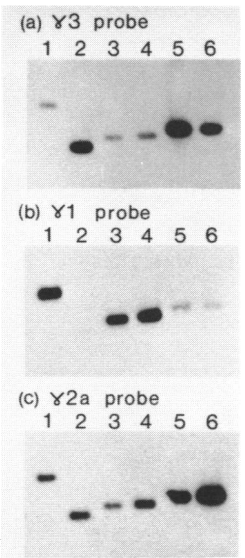

Fig. 1. Identification of the Cγ genes within clones G3.1, G1.1 and G1.2 by hybridization with Cγ3, Cγ1 and Cγ2a probes. The indicated Cγ probes were hybridized to Hind III/Eco RI digests of G1.2 DNA (lane 1) and G1.1 DNA (lane 3); a Xba I digest of G3.1 DNA (lane 2) and Eco RI digests of cDNA plasmids pM21γ1.1 (lane 4), pY5606γ3.15 (lane 5), pM173γ2a.15 (lane 6) (28). The Cγ3 probe was from pY5606γ3.15 and the Cγ2a from pM173γ2a.15 (28). The Cγ1 probe was fragment c in Fig. 6, and a similar result was obtained with a probe from pM21γ1.1. Since 0.03 μg of phage DNA and 0.01 μg of plasmid DNA were loaded, the plasmid sequences are in about 3-fold molar excess. The sizes of the gene-containing fragments are 6.5 kb (lane 1), 4.45 kb (lane 2) and 5.0 kb (lane 3).

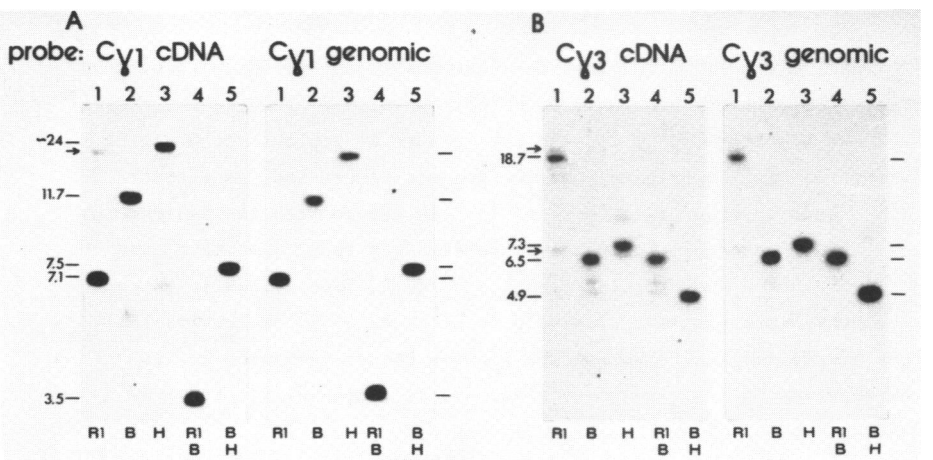

Figure 2. Southern analysis showing that fragments from the genomic clones and authentic Cγ cDNA probes hybridize to the same mouse DNA sequences. The Cγ1 and Cγ3 cDNA probes were from pM21γ1.1 and pY5606γ3.15 (28); the presumptive Cγ1 genomic probe was fragment c from G1.1 in Fig. 6 and the Cγ3 genomic probe was fragment e in Fig. 6. The digests of mouse embryo DNA (15 μg) were made with (1) Eco RI, (2) Bam HI, (3) Hind III, (4) Eco RI + Bam HI, and (5) Bam HI + Hind III. Fragment sizes are given in kb. Faintly labelled Eco RI fragments indicated by arrows correspond to Cγ2-bearing fragments (10).

mouse DNA fragments as the $C_{\gamma 3}$ cDNA probe. Moreover, the $C_{\gamma 1}$ and $C_{\gamma 3}$ patterns are clearly distinct and the fragment sizes differ from the known sizes of those bearing the $C_{\gamma 2a}$ and $C_{\gamma 2b}$ genes (10,16,19,20). Thus the results in Fig. 2 exclude $C_{\gamma 2a}$ and $C_{\gamma 2b}$ as possibilities and leave little doubt that G1.1 is a $C_{\gamma 1}$ clone and G3.1 a $C_{\gamma 3}$ clone.

Restriction maps of G3.1 and two sub-clones are presented in Fig. 3, and maps of G1.1 and G1.2 and subclones in Fig. 4. G3.2 proved to contain the same DNA segment as G3.1, inserted into the vector in the opposite orientation. The maps support the identity of the clones. For G1.1 and G1.2, multiple sites within and around the gene are in complete accord with published maps of a cloned Eco RI fragment bearing the $C_{\gamma 1}$ gene (17,18). Moreover, the gene regions of all the clones lack the Xho I and other sites characteristic of the $C_{\gamma 2a}$ cDNA sequence (28) and the $C_{\gamma 2b}$ gene (19,20).

We positioned the C_{γ} gene within each clone by hybridization (26) to multiple digests like those in Fig. 1. To determine whether the clones contained a second C_H gene, digests were scored with both the different C_{γ} probes and with cloned C_{μ} and C_{α} probes (28). We found no evidence of a second C_H gene. Although several of the probes include V_H and J_H sequences (28), no V_H or J_H sequence was found, in accord with the accumulating evidence (see (1)) that the V_H genes are located in the germline a large but unknown distance from the C_H genes and that all J_H genes are located near the C_{μ} gene (2,4,5, Cory et al., manuscript

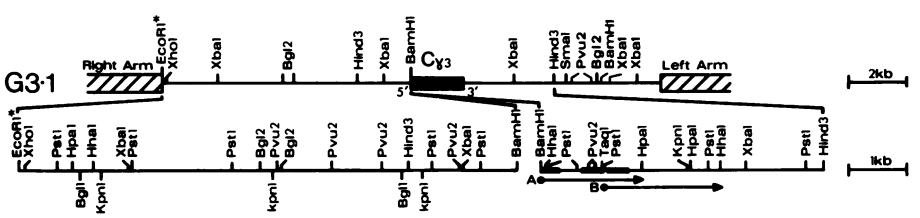

Figure 3. Restriction endonuclease maps of Cγ3 gene-bearing clone G3.1 and two subclones. The upper portion shows the relationship of the Cγ3 gene to the Ch 4A vector arms. Eco RI* indicates Eco RI sites generated by the cloning method (25). The lower portion shows more detailed maps of two subclones in plasmid pBR322. The Cγ3 coding region is denoted by filled boxes. Fragments A and B were used to derive the map in Fig. 6 (see Experimental Procedures). No Bgl I or Bgl II sites were detected in the Bam HI/Hind III subclone and Taq I was not tested on the Eco RI/ Bam HI subclone. No Sal I sites occur in G3.1.

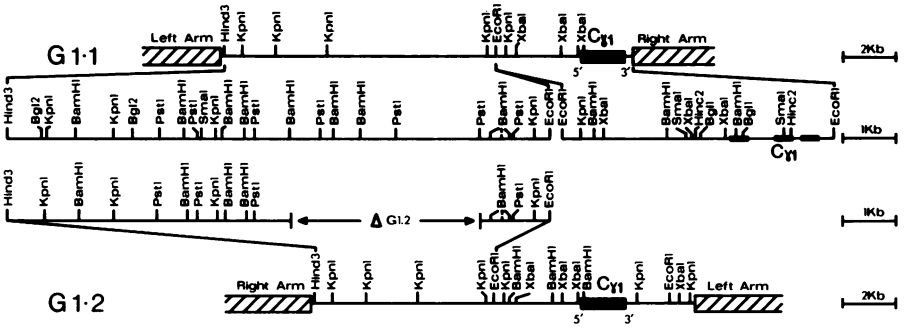

Figure 4. Restriction endonuclease map of the Cγ1-bearing clones G1.1 and G1.2 and of three subclones. Xho I and Sal I did not cleave within these clones. Hind III/Eco RI subclones of G1.1 and G1.2 and an Eco RI subclone of G1.1 are shown. The Hind III/Eco RI subclone of G1.1 is positioned above that of G1.2 to show that a 3.4 kb deletion (Δ G1.2) exists in G1.2 (see text). For these subclones Kpn I, Bgl II, Sma I, Bam HI, Pst I and Hpa I activities were examined, Hpa I making no cleavages. In the Eco RI subclone, the enzymes tested were Hinc 2, Bgl I, Sma I, Kpn I, Bam HI, Xba I, Pvu 2, Pst I and Hpa II, the latter three giving numerous splits which were not mapped.

submitted).

The orientation of the $C_{\gamma 3}$ gene within the mouse DNA insert was established by aligning detailed restriction maps of the gene and $C_{\gamma 3}$ cDNA (see below), which had been aligned with the mRNA by an independent method (28). The $C_{\gamma 1}$ gene was oriented from known restriction sites in the gene region (17,18). Orientations were confirmed by hybridization of a probe specific for the 5' end of the C region (from pM173γ2a.15) (28) to Bam HI and other digests of the clones, and by heteroduplexes between the clones (Tyler and Adams, manuscript submitted).

The $C_{\gamma 3}$ gene is divided into domain-like segments.

To determine whether the $C_{\gamma 3}$ gene contains intervening sequences, we constructed detailed restriction maps of the gene region by partial digestion (31) of two fragments spanning the gene (A and B in Fig. 3) (see Experimental Procedures). Fig. 5 compares this map with that previously determined (28) for $C_{\gamma 3}$ cDNA. Regions where the two maps agree, indicated by stippling, represent coding segments (C1-C3), whereas regions where the patterns differ represent intervening sequences (I1-I3). The results indicate that the $C_{\gamma 3}$ gene contains three coding segments of domain size and probably also a hinge region, as in other C_{γ} genes (see

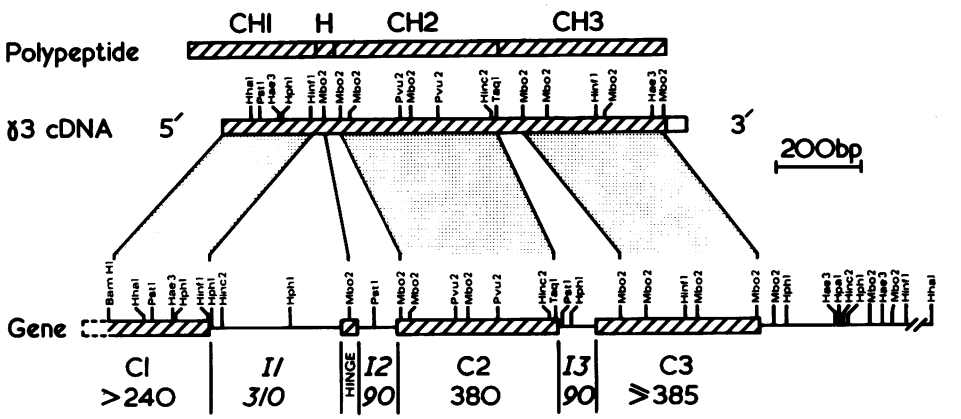

Figure 5. Restriction maps of γ3 cDNA and the Cγ3 gene demonstrating that the gene is divided into domain-like segments. The cDNA segment in clone pγ5606γ3.15 (28) is compared with the Cγ3 gene region in G3.1. Restriction sites which define the boundaries of the coding sequences are related to equivalent sites in the cDNA restriction map by solid lines. Estimated sizes of the coding regions (C1-C3) and intervening sequences (I1-I3) are given in base pairs. Region C1 can be related to the cDNA restriction map as far 5' as the cDNA insert extends. Region C3 corresponds to the cDNA up to the rightmost Mbo II site, except for the rightmost Hae III site, which may reflect a cloning aberration.

Discussion).

No large common sequences occur 5' to C_H genes.

Since the entire C_H locus is believed to be derived from a single ancestral C_H gene and the sequences flanking each C_H gene are implicated in switch recombination, different C_H flanking regions might contain common sequences. To test this, we used sequences 5' to the cloned $C_{\gamma 1}$ and $C_{\gamma 3}$ genes as probes to score restriction digests of mouse DNA. If the sequences 5' to each C_H gene contained a common element, eight fragments should hybridize. Instead, Fig. 6 shows that each probe appeared to label a unique sequence. Thus, probes a and b from G1.1, which together represent the entire cloned 5' flanking sequences extending 0.3 kb to 13.0 kb from the $C_{\gamma 1}$ gene, each labelled a single fragment, in more than one digest (lanes 1-5). Similarly, the 5' flanking sequence in G3.1 (probe d) labelled a single Eco RI fragment (lane 6) and Bam HI fragment (lane 8) and two Hind III fragments (lane 7), as expected from the Hind III site within the probe region.

We conclude that no large closely related sequences precede each C_H

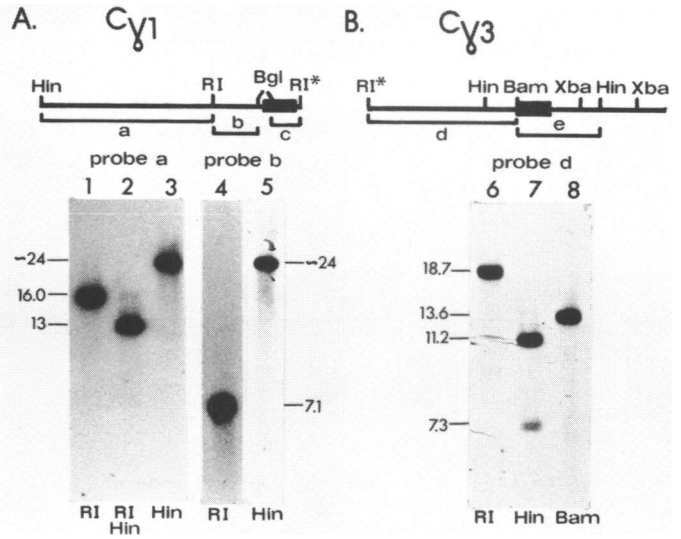

Figure 6. Southern analysis showing that sequences 5' to the Cγ1 and Cγ3 genes behave like unique sequences. The indicated probes were hybridized to fragments of mouse embryo DNA generated with EcoRI (lanes 1,4,6), Eco RI + Hind III (lane 2), Hind III (lanes 3,5,7) and Bam HI (lane 8). Fragment sizes are indicated in kb. The Hind III, Eco RI, Bgl 1, and Bam HI sites that define the probes are indicated (see Figs. 3 and 4).

gene. In view of the very strong signals obtained in Fig. 6, any closely homologous sequence more than about 150 bp long should have been revealed. Since stringent hybridization conditions were used (equivalent to Tm-14°), we would not expect to detect regions having less than about 80% homology with the probe. Heteroduplex analysis indicates that the $C_{\gamma 1}$ and $C_{\gamma 3}$ 5' flanking sequences do exhibit about 40-55% homology (Tyler and Adams, manuscript submitted).

Deletions in the 5' flanking region of two independent $C_{\gamma 1}$ clones.

Comparison of the maps of G1.1 and G1.2 suggested that G1.2 had suffered a 3.4 kb deletion in the 5' flanking region (Fig. 4). Moreover, heteroduplexes formed between subclones from G1.1 and G1.2 exhibited a deletion loop, as illustrated in Fig. 7. Measurements on 12 molecules indicated that the deletion loop was 3.84 ± 0.53 kb (S.E.) long and that it was located 5.06 ± 0.41 kb (S.E.) 3' to the Hind III site in the

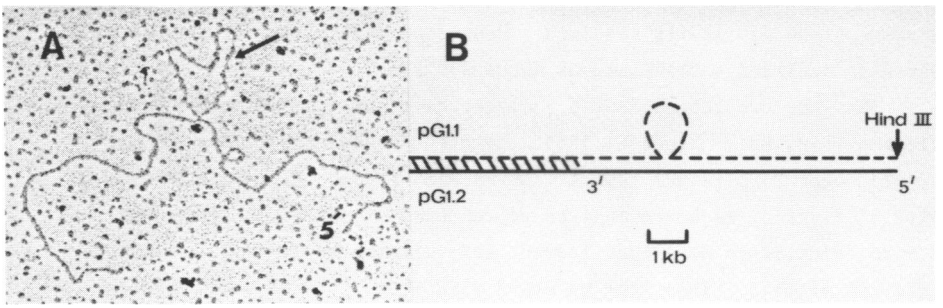

Figure 7. Heteroduplex analysis showing that a segment of the G1.1 5' flanking sequence is absent from G1.2. Heteroduplexes were formed between Hind III/Eco RI subclones of G1.1 and G1.2 which had been cleaved with Hind III and a typical molecule is shown with the deletion loop indicated by an arrow. In the diagram plasmid sequences are hatched and the 5' Hind III site of the insert is indicated.

insert. Comparison of Bam HI and Pst sites in the two subclones indicates that the deletion starts 4.6 kb 5' to the $C_{\gamma 1}$ gene (Fig. 4).

To determine whether the G1.1 flanking sequence had also undergone a deletion, we compared the size of the Hind III-Eco RI fragment in embryo DNA with that from G1.1 directly by Southern analysis. Fig. 8A shows that the fragment from the clone (10.1 kb in lane 1) is distinctly

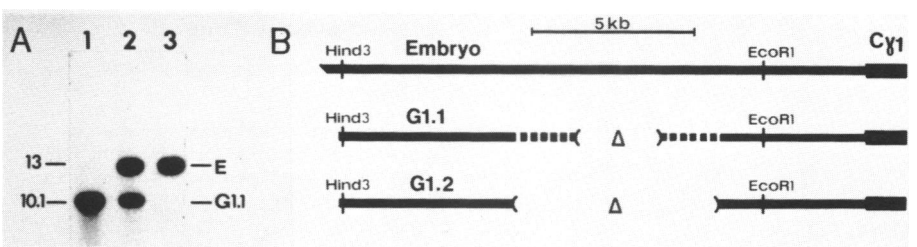

Figure 8. Deletions in two Cγ1-bearing clones.
A. Southern experiment showing that the 5' Hind III/Eco RI fragment of G1.1 is shorter than the corresponding region in embryo DNA. Fragment a in Fig. 6 was hybridized to Hind III/Eco RI digests of (1) G1.1 DNA (104 pg), (2) embryo DNA (15 µg) + G1.1 DNA (52 pg), and (3) embryo DNA alone (15 µg). The amount of cloned fragment in lane 2 relative to embryo DNA corresponds to 1 copy per haploid genome, assuming that the haploid mouse genome contains 2.9×10^6 kb (36).
B. Relation of the cloned sequences to those in the genome. Both clones have suffered deletions within the indicated regions during cloning.

smaller than that present in embryo DNA (13 kb in lane 3) and that a mixture (lane 2) readily resolved. Hence the G1.1 5' flanking sequence has also suffered a deletion, of about 2.9 kb.

Thus the two independent $C_{\gamma 1}$ clones have suffered deletions during cloning (Fig. 8B). Since G1.1/G1.2 heteroduplexes exhibited a single displacement loop rather than a loop on each strand (Fig. 7), the entire G1.2 5' flanking sequence must be represented within G1.1; hence the 2.9 kb deletion in G1.1 must lie entirely within the 2.9 + 3.4 = 6.3 kb deletion of G1.2. Thus, the deletion within G1.1 starts somewhere between 4.6 and 5.1 kb from the $C_{\gamma 1}$ gene. Cloning deletions can result from repetitive sequences (34). The deletions within the same region in two independent $C_{\gamma 1}$ clones probably reflect the presence of the repetitiv sequences in this region, which may be involved in switch recombination (7,35; Tyler & Adams, manuscript submitted).

We found no evidence that G3.1 has suffered a deletion. Mixing experiments like those in Fig. 8 indicated that fragments d and e from G3.1 (Fig. 6) are the same size as those from genomic DNA. Since the independent clones G3.1 and G3.2 yield restriction fragments of identical sizes, any sizable deletion during cloning is very unlikely.

Linkage of the $C_{\gamma 3}$ and $C_{\gamma 1}$ genes.

In an attempt to establish linkage of the $C_{\gamma 3}$ and $C_{\gamma 1}$ genes, we used probes derived from the $C_{\gamma 3}$ gene (or the region 3' to it) and the region 5' to the $C_{\gamma 1}$ gene to score fragments of mouse embryo DNA made with a number of restriction endonucleases. Fig. 9 shows that in a Bgl 1 digest a $C_{\gamma 3}$ probe (a in Fig. 10) revealed a large fragment (36-42 kb long) which had the same mobility as one labelled by probes

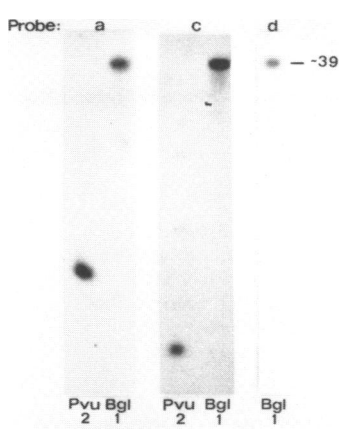

Figure 9. Southern analysis showing a probable overlap between the Cγ3 and Cγ1 genes. The indicated probes, defined in Fig. 10, were hybridized to Pvu 2 and Bgl 1 digests of embryo DNA. The Pvu 2 digests show that probes a and c are derived from distinct DNA regions.

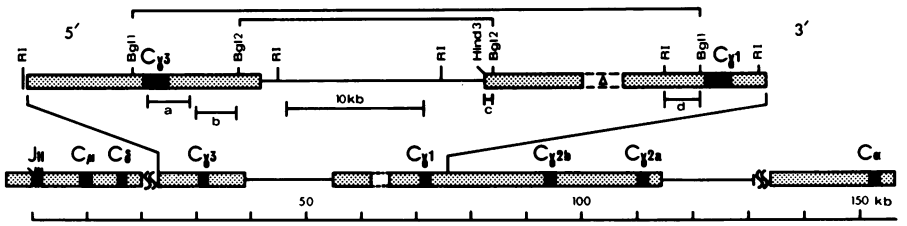

Figure 10. The region around the Cγ3 and Cγ1 genes and its relationship to the C_H locus. The top map shows restriction sites that support the proposed Cγ3-Cγ1 spacing, with cloned sequences shown stippled. Fragments used as probes (see Figs. 3 and 4) were a, a Hha fragment from the Bam/Hind III subclone of G3.1; b, the larger Xba fragment 3' to the Cγ3 gene, subcloned in pBR322; c, the 5' Hind III/ Kpn I fragment from the Hind III/Eco RI subclone of G1.1; and d, an Eco RI/Bgl I fragment from the Eco RI subclone of G1.1. At the bottom, the proposed Cγ3-Cγ1 linkage is related to published data on Cμ (22,23), Cδ (14), Cα (6) and the Cγ1-Cγ2b (15,16) and Cγ2b-Cγ2a linkages (16). The minimal spacing for the Cγ2a and Cα genes is based on the occurrence of the Cγ2a gene in an ~22 kb Eco RI fragment (10).

from the extreme 5' end of the cloned $C_{\gamma 1}$ flanking region (fragment c in Fig. 10) and from just 5' to the $C_{\gamma 1}$ gene (d in Fig. 10); the large Bgl 1 fragment was also labelled by a probe 3' to the $C_{\gamma 3}$ gene (b in Fig. 10). To confirm that these fragments were indistinguishable in size, a filter previously scored with the $C_{\gamma 3}$ probe was rehybridized with fragment c and a single band was found. These results suggest that the $C_{\gamma 3}$ and $C_{\gamma 1}$ genes are linked, with a spacer region of about 33-39 kb (Fig. 10).

Bgl 2 results (not shown) provided further information on the spacing. Fragment a of Fig. 10 labelled an 18.3 kb Bgl 2 fragment. As indicated in Fig. 10 this observation is consistent with the Bgl 1 results if the Bgl 2 fragment abuts the most 3' Bgl 2 site of G3.1. (The 18.3 kb Bgl 2 fragment should then be labelled by a probe from the extreme 3' end of G3.1 but we could not confirm this because this 3' region contains sequences that are highly reiterated in the genome.) Thus our data suggests that the $C_{\gamma 3}$ gene is 37-40 kb 5' to the $C_{\gamma 1}$ gene (Fig. 10).

DISCUSSION

The $C_{\gamma 3}$ gene has a domain structure similar to that of the $C_{\gamma 2b}$ and $C_{\gamma 1}$ genes.

Fig. 11 compares the structure proposed here for the $C_{\gamma 3}$ gene with those established for the $C_{\gamma 2b}$ (19,20) and $C_{\gamma 1}$ (17,18) genes. The similarity is striking: within the error of our measurements (± 35 bp), the position and length of the intervening sequence is the same for the three C_{γ} genes. This suggests that the intervening sequences were present within a proto-C_{γ} gene before divergence of the C_{γ} subclasses. Comparison of the restriction maps indicates however that the nucleotide sequence within both the coding and intervening regions have diverged considerably. Most prominent among the sites which appear to be preserved (shown bold) is the Hinc 2-Taq-Pst cluster near the end of domain 2. Yamawaki-Kataoka et al. (20) have suggested that the nucleotide sequence in this region is conserved to maintain a proper splicing site. This might also account for the conserved Hph I site just 3' to domain 1. Heteroduplex analysis indicates that the $C_{\gamma 3}$ and $C_{\gamma 1}$ intervening sequences have diverged much more than their coding sequences (Tyler & Adams, manuscript submitted). This is consistent with the notion that a precise sequence within an intervening region is not required for any function of that region, or for its excision.

$C_{\gamma 3}$ and $C_{\gamma 1}$ are single-copy genes.

The Southern analyses reported here strongly suggest that the $C_{\gamma 3}$ and the $C_{\gamma 1}$ gene occur as a single copy per haploid mouse genome. Both the genes themselves (Fig. 2) and their flanking sequences extending 8-13 kb 5' to the genes (Fig. 6) behaved like unique sequences in multiple

Figure 11. Structure of the Cγ3, Cγ1, and Cγ2b genes. The Cγ3 gene from mouse embryo is compared with the Cγ1 gene (17,18) and Cγ2b gene (19,20). Coding segments are shown as bars, intervening sequences as lines and common restriction sites are shown in bold.

digests. Moreover, the results from a mixing experiment in which cloned $C_{\gamma 1}$ flanking DNA was added to embryo DNA at the single copy level (Fig. 8) suggests that this region is represented once per haploid genome, and a similar experiment with the $C_{\gamma 3}$ 5' flanking sequence (not shown) led to the same conclusion. The evidence for $C_{\gamma 3}$-$C_{\gamma 1}$ linkage (Figs. 9,10) also favors these genes being unique. Our Southern data also makes it very unlikely that the mouse genome contains previously undiscovered C_{γ} genes closely related to $C_{\gamma 1}$ or $C_{\gamma 3}$ (analogous to $C_{\gamma 2a}$ and $C_{\gamma 2b}$), because genes as closely related as $C_{\gamma 2a}$ and $C_{\gamma 2b}$ readily cross hybridize under our conditions (10).

The C_H locus is at least 150 kb long.

Our data suggest that the $C_{\gamma 3}$ gene lies 37-40 kb 5' to the $C_{\gamma 1}$ gene. This spacing is much larger than the 21 kb between $C_{\gamma 1}$ and $C_{\gamma 2b}$ (15,16), the 15 kb between $C_{\gamma 2b}$ and $C_{\gamma 2a}$ (16) and the 2 kb between C_{μ} and C_{δ} (14). The differences in C_{γ} spacing may reflect the evolution of the locus, since the $C_{\gamma 2a}$ and $C_{\gamma 2b}$ genes are most closely homologous while $C_{\gamma 1}$ and $C_{\gamma 3}$ are the most divergent C_{γ} genes (33). Combining our results with all the published data (Fig. 10) indicates that the C_H locus is much larger than might have been expected, spanning at least 150 kb. Considering that the C_{ε} gene has not yet been placed and that the $C_{\gamma 2a}$-C_{α} and C_{δ}-$C_{\gamma 3}$ distances are unknown, it seems likely that the locus is between 175 and 250 kb long.

ACKNOWLEDGMENTS

We are grateful to T. Honjo for manuscripts, to Brett Tyler for discussions and to Jill Gough and Jan Holton for skilled technical assistance. This work was supported by the U.S. National Cancer Institute (RO1-CA12421), the American Heart Association, and the N.H. and M.R.C. (Canberra).

REFERENCES

1. Adams, J.M. (1980) Immunology Today 1, 10-17
2. Early, P.W., Davis, M.M., Kaback, D.B., Davidson, N. and Hood, L. (1980) Cell 19, 981-992
3. Cory, S., Adams, J.M. and Kemp, D.J. (1980) Proc. Acad. Sci. U.S.A., 77, 4943-4947
4. Gough, N.M. and Bernard, O. (1980) Proc. Natl. Acad. Sci. U.S.A., in press
5. Sakano, H., Maki, R., Karosawa, Y., Roeder, W. and Tonegawa, S. (1980) Nature 286, 676-683
6. Davis, M.M., Calame, K., Early, P.W., Livant, D.L., Joho, R., Weissman, I.L. and Hood, L. (1980) Nature 283, 733-739

7. Kataoka, T., Kawakami, T., Takahashi, N. and Honjo, T. (1980) Proc. Nat. Acad. Sci. U.S.A. 77, 919-923
8. Cory, S. and Adams, J.M. (1980) Cell 19, 37-51
9. Honjo, T. and Kataoka, T. (1978) Proc. Nat. Acad. Sci. U.S.A. 75, 2140-2144
10. Cory, S., Jackson, J. and Adams, J.M. (1980) Nature 285, 450-455.
11. Coleclough, C., Cooper, D. and Perry, R.P. (1980) Proc. Nat. Acad. Sci. U.S.A. 77, 1422-1426
12. Rabbitts, T.H., Forster, A., Dunnick, W. and Bentley, D.L. (1980) Nature 283, 351-356
13. Yaoita, Y. and Honjo, T. (1980) Biomedical Res. 1, 164-175
14. Blattner, F. and Newell, N. (1980) Cold Spring Harbor Symposium, in press
15. Maki, R., Traunecker, A., Sakano, H., Roeder, W. and Tonegawa, S. (1980) Proc. Nat. Acad. Sci. U.S.A. 77, 2138-2142
16. Honjo, T., Kataoka, T., Yaiota, Y., Shimizu, A., Takahashi, N., Yamawaki-Kataoka, Y., Nikaido, T., Nakai, S., Obata, M., Kawakami, T. and Nishida, Y. (1980) Cold Spring Harbor Symposium, in press
17. Sakano, H., Rogers, J.H., Hüppi, K., Brack, C., Traunecker, A., Maki, R., Wall, R. and Tonegawa, S. (1979) Nature 277, 627-633
18. Honjo, T., Obata, M., Yamawaki-Kataoka, Y., Kataoka, T., Kawakami, T., Tahakashi, N. and Mano, Y. (1979) Cell 18, 559-568
19. Tucker, P.W., Marcu, K.B., Newell, N., Richards J. and Blattner, F.R. (1979) Science 206, 1303-1306
20. Yamawaki-Kataoka, Y., Kataoka, T., Takahashi, N., Obata, M. and Honjo, T. (1980) Nature 283, 786-789
21. Early, P.W., Davis, M.M., Kaback, D.B., Davidson, N. and Hood, L. (1979) Proc. Nat. Acad. Sci. U.S.A. 76, 857-861
22. Gough, N.M., Kemp, D.J., Tyler, B.M., Adams, J.M. and Cory, S. (1980) Proc. Nat. Acad. Sci. U.S.A. 77, 554-558
23. Calame, K., Rogers, J., Early, P., Davis, M., Livant, D., Wall, R. and Hood, L. (1980) Nature 284, 452-455
24. Kawakami, T., Tahahashi, N. and Honjo, T. (1980) Nucleic Acids Res. 8, 3933-3945
25. Kemp, D.J., Cory, S. and Adams, J.M. (1979) Proc. Nat. Acad. Sci. U.S.A. 76, 4627-4631
26. Southern, E.M. (1975) J. Mol. Biol. 98, 503-517
27. Benton, W.D. and Davis, R.W. (1977) Science 196, 180-182
28. Adams, J.M., Gough, N.M., Webb, E.A., Tyler, B.M., Jackson, J. and Cory, S. (1980) Biochemistry 19, 2711-2719
29. Rigby, P.J.W., Dieckmann, M., Rhodes, C. and Berg, P. (1977) J. Mol. Biol. 113, 237-251
30. Jeffreys, A.J. and Flavell, R.A. (1977) Cell 12, 429-439
31. Smith, H.O. and Birnstiel, M.L. (1976) Nucleic Acids Res. 3, 2387-2398
32. Tyler, B.M. and Adams, J.M. (1980) Gene 10, 147-155
33. Yamawaki-Kataoka, Y., Sato, K., Shimizu, A., Kataoka, T., Mano, Y., Ono, M., Kawakami, M. and Honjo, T. (1979) Biochemistry 18, 490-494
34. Brutlag, D., Fry, K., Nelson, T. and Hung, P. (1977) Cell 10, 509-519
35. Dunnick, W., Rabbitts, T.H. and Milstein, C. (1980) Nature 286, 669-675
36. Laird, C.D. (1971) Chromosoma 32, 378-406