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Comparison of the hinge-coding segments in human immunoglobulin gamma heavy chain genes and the linkage of the gamma 2 and gamma 4 subclass genes

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The genes for the human immunoglobulin heavy chain constant region subclasses have been examined in clones isolated from a phage library containing human genomic DNA. Nucleotide sequencing and restriction enzyme mapping show that the CH1, hinge, CH2, and CH3 domains are present as distinct genetic elements separated by short intervening sequences in these genes: the γ3 gene is unique amongst these genes in that it possesses four separate hinge-coding segments. A further γ gene has been identified which does not correspond to a known human γ protein: this gene contains a hinge related to the first hinge of the γ3 gene and may represent an inactive or pseudo γ gene. Comparison of a number of overlapping clones containing γ2 and γ4 genes shows that they are separated in human DNA by ~19 kb and that the γ2 gene is located upstream of γ4. Both genes (order 5′Cγ2-Cγ4-3′) are orientated in the same direction of transcription.
Key words: antibody genes/gammaglobulins/gene linkage/heavy chain genes

Introduction

The genes encoding human immunoglobulin heavy chains are encoded on chromosome 14 comprising a linked family of variable (VH) region and constant (CH) region genes (Croce *et al.*, 1979; Hobart *et al.*, 1981). Within the CH locus there are five classes of CH gene (μ, δ, γ, ϵ, and α) which define the type of antibody expressed by a lymphocyte (IgM, IgD, IgG, IgE, and IgA, respectively). Furthermore, subclasses are known for the gammaglobulins (IgG1, IgG2, IgG3, and IgG4) and IgA (IgA1 and IgA2) (reviewed by Natvig and Kunkel, 1973). During expression, a VH gene is initially expressed with Cμ but later a single B-lymphocyte expresses this same V gene with different CH genes after the so-called H-chain class switch (Cooper *et al.*, 1976). A map of the physical linkage and sequence relationship between the various CH genes is necessary for our understanding of the mechanism involved in this class switch. So far little information is available on the human genomic segment encoding the CH region genes although an isolated clone has been described which contains the human Cμ and the Cδ genes separated by 5 kb of DNA (Rabbitts *et al.*, 1981). Here we present the analysis of four human Cγ genes in DNA segments from a library of human foetal liver DNA clones in Charon 4A lambda phages. The cloned Cγ genes were classified by nucleotide sequence analysis, particularly of the hinge-coding segments, and mapping utilising restriction endonuclease sites. The domain structure of the human γ genes shows, like those of mouse, separate coding segments and in addition the human γ3 has four separate hinge-coding segments. A contiguous map of DNA carrying the Cγ2 and Cγ4 genes shows that Cγ4 is located 19 kb downstream of the Cγ2 gene. Both genes lie in the same orientation of transcription.

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Results and Discussion

It has been shown previously that cDNA clones which contain either the coding region of mouse VH segments or the Cμ gene, will cross-hybridise, in low stringency conditions, to the equivalent genes in human foetal liver DNA (Matthyssens and Rabbitts, 1980). We also found weak but detectable hybridisation between mouse γ1 sequences and a related gene in human DNA (data not shown). We have utilised this cross-hybridisation between immunoglobulin heavy chain genes of mouse and man to isolate Cγ genes from the human genome using a cloned cDNA plasmid containing mouse γ1 sequences (pγ1/A5) (Dunnick *et al.*, 1980). By screening 10^6 plaques of a human foetal liver DNA phage library (Lawn *et al.*, 1978) we obtained one clone (λHG4.6) which hybridised to the radioactive mouse γ1 plasmid. A 3-kb *Pst*I fragment of λHG4.6, carrying the human Cγ coding segments, was subcloned in pBR322 giving a plasmid designated pγ2P. This 3-kb *Pst*I fragment contained 1.2 kb of human genomic DNA and 1.8 kb of λ Charon 4A vector DNA separated by an *Eco*RI site (Figure 1A). A further subclone (pγ2RPA3) was generated from pγ2P by cloning the 1.95-kb *Eco*RI fragment in pACYC184; this *Eco*RI fragment contained the 1.2-kb *Eco*RI-*Pst*I fragment of human DNA plus the 0.75-kb *Pst*I-*Eco*RI fragment of pBR322 (Figure 1A).

The 1.2-kb human DNA fragment hybridised to mouse Cγ probes and therefore contains human Cγ coding segments. To determine the subclass of the human Cγ gene, the *Eco*RI-*Pst*I fragment was cloned in M13mp8 and mp9 (Messing *et al.*, 1981) and nucleotide sequences were derived using the dideoxy chain termination procedures (Sanger *et al.*, 1977). The nucleotide data thus obtained identified the gene as the γ2 subclass gene (Figure 1B). The *Pst*I site (CTGCAG) represents the beginning of the hinge sequence (a region made of 12 codons) and the derived amino acid sequence shows a single discrepancy in the hinge region of the cloned gene compared to the SA γ2 protein (Frangione *et al.*, 1969); the gene codes for serine rather than cysteine in the penultimate position. This difference may represent a genetic polymorphism at this site. The hinge is separated by intervening sequences (IVS) from the CH1 domain on the 5′ side and the CH2 domain on the 3′ side, the CAG triplet of the *Pst*I being the RNA splicing signal at the 3′ end of the IVS between the CH1 and hinge domains. The hinge and CH2 coding segments are separated by an IVS of 116 bases and, in keeping with other immunoglobulin genes, the first codon of both hinge and CH2 domains are created by RNA splicing. The 3′ end of the CH3 domain was located by nucleotide sequencing 180 bases from the left-hand *Eco*RI site (Figure 1B). The derived sequence of 17 amino acids terminates with a UGA

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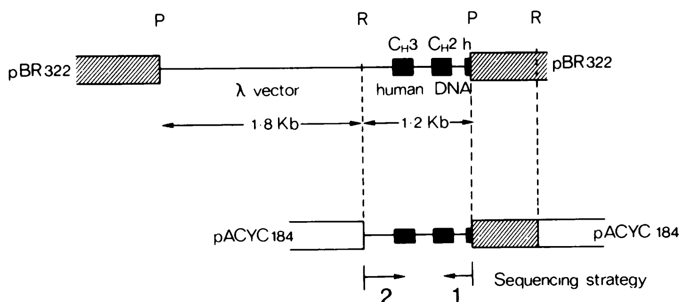

Sequence 1

```
     E   R   K   C   C   V   E   C   P   P   S   P
CTGCAGAGCGCAAATGTTGTGTCGAGTGCCCACCGTCCCCAGGTAAGCCAGCCCAGGCCT
                                                          60

GCCCTCCAGCTCAAGGCGGGACAGGTGCCCTAGAGTAGCCT-CATCCAGGGACAGGCCCC
                                                         120

                                         A   P   P   V   A   G   P   S
AGTTGGGTGTTGACA-GTCCACCTCCATCTCTTCCTCAGCACCACCTGTGGCAGGACCGT
                                                         180

  V   F   L   F   P   P   K   P   K   D   T   L   M   I   S   R   T   P   E   V
CAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGG
                                                         240

  T   C   V   V   V   D   V   S   H   E   D   P   E   V   Q   F   N   W   Y   V
TCACGTGCGTGGTGGTGGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACG
                                                         320

  D   G   V   E   V   H   N   A   K   T   K   P   R   E   E   Q   F   N   S
TGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCA
                                                         360
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Sequence 2.

```
 A   L   H   N   H   Y   T   Q   K   S   L   S   L   S   P   G   K   *
GCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGAATCCAC
                                                          60

GGCCGCAAGCCCCCACTCCCCA-CTCTCGGGGGCACGTGAGCATGCTTGGCACGTACCCC
                                                         120

GTGTACATACTTCCCAGGGACCCAGCATGGAAATAAAGACCCAGGCTGCCCTGGGCCCTG
                                                         180

CGA
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Fig. 1. Structure of human γ2 probe and partial DNA sequence data of γ2 coding segment. **(A)** Plasmid pγ2P contains a 3-kb *Pst*I fragment from λHG4.6 subcloned in pBR322. This fragment contains a 1.8-kb *Eco*RI-*Pst*I fragment from vector λ Charon 4A and a 1.2-kb fragment of human DNA containing hCH2CH3 region of Cγ2 gene. From pγ2P, the 1.9-kb *Eco*RI fragment was recloned in pACYC184 to generate pγ2RPA3. P = *Pst*I, R = *Eco*RI. **(B)** Nucleotide sequence data was generated by cloning the *Eco*RI-*Pst*I fragment in M13mp8 (sequence 1) or mp9 (sequence 2) and sequencing by the dideoxy chain termination method. Amino acids are given in the one letter code and the star (sequence 2) represents the chain termination codon. RNA splicing sites are indicated by arrows and the *Pst*I restriction enzyme site of the 5′ end of sequence 1 is underlined. The AATAAA sequence characteristic of poly(A) addition sites is boxed.

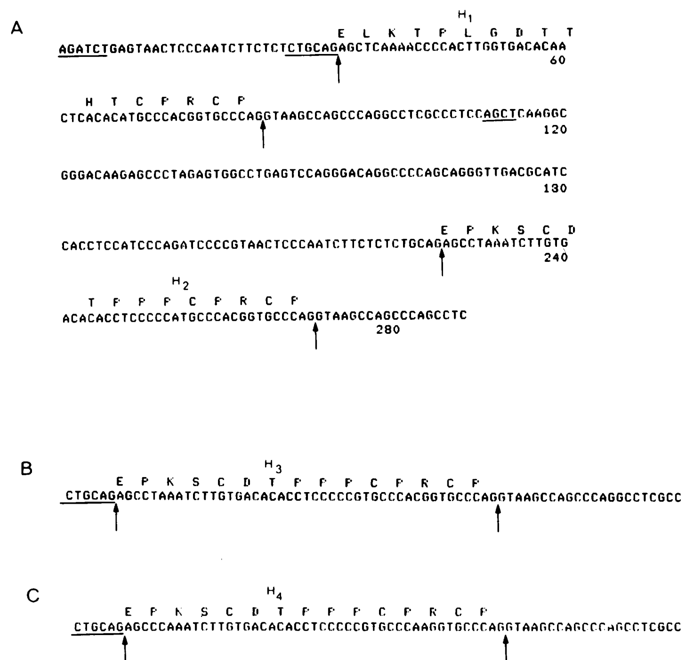

Fig. 2. Partial nucleotide sequence of hinge region of Cγ3 gene. An *Eco*RI-*Hind*III fragment (3.6 kb) containing the γ3 coding segment from λHG4.2 was subcloned in pBR322 and various clones made from this in M13 vectors for sequencing by the dideoxy chain termination method. M13 clones containing coding segments were identified, after sequencing, by homology to the γ3 protein sequence. The derived amino acid sequences are given in the one letter code. RNA splicing sites are indicated by arrows and the first codon of each hinge segment is created after these splicing events. **(A)** Hinge coding segments 1 and 2 plus IVS. *Bgl*II, *Pst*I, and *Alu*I fragments were cloned in M13mp9 and fragments were sequenced in both orientations: the relevant sites are indicated by underlining. **(B)** Hinge coding segment 3. A *Pst*I fragment was cloned in M13mp9: sequencing was from the *Pst*I site (underlined). **(C)** Hinge coding segment 4. *Pst*I-*Eco*RI fragments were cloned in M13mp8 and sequenced away from the *Pst*I site (underlined). The identification of this fragment as the h_4 segment comes from its location nearest to the 3′ *Eco*RI site of λHG4.2. H_1, H_2, H_3, and H_4 refer to the four hinge-coding segments.

codon and agrees with the γ2 protein PIG (Pardo *et al.*, 1978) except for the presence of a terminal lysine codon in the gene which is not present in the serum gammaglobulin. A similar situation has been described for the mouse γ1 gene (Dunnick *et al.*, 1980; Honjo *et al.*, 1979) and therefore it is highy probable that the lysine residue is cleaved after translation, as suggested for a human epsilon heavy chain (Bennich *et al.*, 1973). Indeed, we have now shown that the human epsilon CH gene has a lysine codon preceding the chain termination codon (J. Flanagan and T.H. Rabbitts, in preparation). Finally, the nucleotide sequence AATAAA, which characterises the region of poly(A) addition to mRNA transcripts (Proudfoot and Brownlee, 1976), occurs 98 residues from the chain termination codon in this γ2 gene.

Since the nucleotide sequencing enabled us to locate the 3′ end of the CH3 donain, the 5′ end of this domain must occur ~440 bases from the *Eco*RI site. The CH2 and CH3 domains of the γ2 gene are therefore separated by an IVS of ~200 bases. The domain structure of the human γ2 gene is, therefore, similar to that first described for the mouse Cγ1 gene (Sakano *et al.*, 1979) and is similar to the human Cμ gene although the latter possesses no distinct hinge domain (Rabbitts *et al.*, 1981; Takahashi *et al.*, 1980). Partial nucleotide data on the γ4 gene given in Figure 2 shows an arrangement for this gene similar to the γ2 subclass gene. The human Cϵ gene also has four domains separated by short IVS (J. Flanagan and T.H. Rabbitts, in preparation) so it is, therefore, probable that the domains described in heavy chain proteins also occur as discrete genetic elements in all human CH genes.

The recombinant plasmid (pγ2RPA3) was used to rescreen the human foetal liver phage library for more Cγ clones. Approximately 10^7 plaques of the phage library were screened by hybridisation with the radioactive 1.2-kb *Eco*RI-*Pst*I fragment of pγ2RPA3 (specific activity 2 x 10^7 c.p.m./μg DNA). We obtained 18 phage clones which hybridised to the Cγ2 probe. Phage DNA was isolated from these clones and mapping of restriction endonuclease sites was carried out. Four groups of clones could be distinguished on the basis of characteristic restriction enzyme sites and partial nucleotide sequence: from these clones, groups were found which contained human Cγ2 (λHG1.4, 4.6, 6.3, 9.6, and 11.4), Cγ3 (λHG4.2), and Cγ4 (λHG2.1, 4.1, and 6.6) genes.. A further clone (λHG12.2) was identified which contains a previously unidentified γ gene. The hinge sequences of the human γ proteins are particularly characteristic of the subclasses, especially the γ3 protein which contains a much longer hinge region than the other subclasses (Edelman *et al.*, 1969; Frangione *et al.*, 1969; Frangione and Franklin, 1979; Pink *et al.*, 1970). The subclass contained in the various groups of clones was, therefore, examined by nucleotide sequencing of the hinge coding segments. Figures 2 and 3 show these data.

The hinge-coding region of the γ3 gene is made of four distinct segments separated from each other by short IVS (Figure 2). This arrangement is distinct from the other γ genes in that these genes possess only one hinge segment (Figures 1 and 3) but there is a high degree of homology between the nucleotide sequences within each hinge segment. The sequence of the γ4 hinge (determined from *Pst*I subclones of

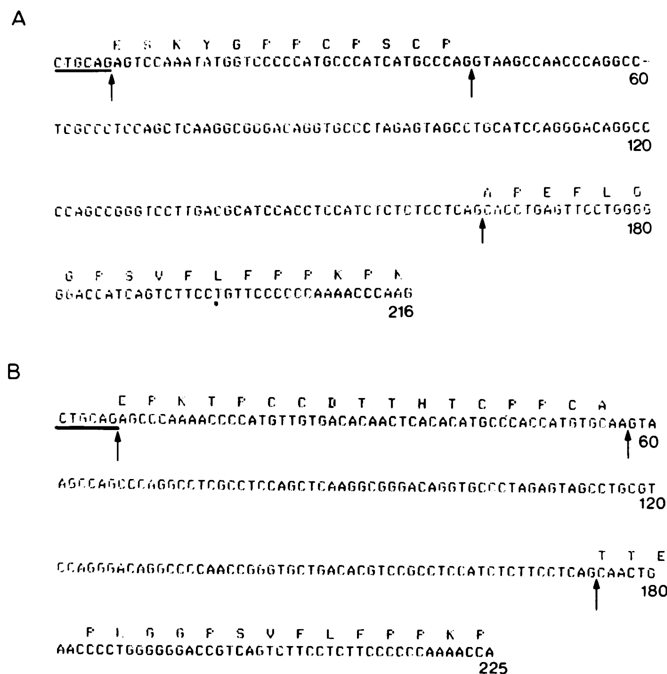

Fig. 3. Partial DNA sequence of Cγ4 and presumptive $\psi\gamma$ hinge and CH2 domains. **(A)** Cγ4. A 1.7-kb *Pst*I fragment containing part of the gamma coding region was cloned in M13mp7 and the nucleotide sequence derived by the dideoxy chain termination procedure. The *Pst*I site at the 5′ end of the fragment is underlined. RNA splicing sites are shown by vertical arrows. Amino acids are given in the one letter code and the first codon of each domain is that created by the splicing. **(B)** Presumptive $\psi\gamma$. A *Pst*I fragment from λHG12.2, cloned in M13mp9, was selected by hybridisation with Pγ2RPA3 and the nucleotide sequence was determined from the *Pst*I site using the dideoxy chain termination procedure. The RNA splicing between hinge and CH2 domains creates the codon for Thr at the beginning of the CH2 domain.

λHG4.1 in M13) together with the IVS and beginning of the CH2 domain are shown in Figure 3. The hinge sequence agrees with the γ4 heavy chain VIN (Pink *et al.*, 1970) except for the presence of Ser instead of Pro at position 10 which could also possibly be due to genetic polymorphism in this region. In the γ4 gene the hinge and CH2 domains are separated from each other by an IVS of 121 nucleotides and again the first codon of each domain is created, in the formation of mRNA, by the RNA splicing.

Figure 3 also shows the sequence of the hinge and start of the CH2 domains of the γ gene from λHG12.2; this sequence does not correspond to any known human γ protein and, in addition, the RNA splice signal (AGT) at the 3′ end of the hinge does not correspond to the sequence (GGT) found at this position in the other γ genes. It seems likely that this gene represents an inactive or pseudo-gene. This γ gene has only a single hinge coding segment and it is related to the first hinge-coding segment of the γ3 gene (87% homology). This homology is particularly striking in the presence of a 6-nucleotide insert, which would code for Thr-Pro, occurring at codon 4 of both sequences but which is absent from γ2 and γ4 hinges (and which would not be expected in the γ1 hinge on the basis of the known protein sequence). This homology argues for an evolutionary relationship between the γ3 gene and the presumptive pseudo γ gene.

Our nucleotide sequence data show that a *Pst*I endonuclease site is part of the splice signal (Breathnach *et al.*, 1978) at the 5′ end of the hinge-coding segment in both Cγ2 and Cγ4 (see Figures 1 and 2). We utilised the position of this *Pst*I site to determine the orientation of cloned Cγ genes (Figures 4 and 5). DNA preparations of various phage clones were digested with *Pst*I; the resulting fragments were separated on 1.5% agarose gels and blotted on nitro-cellulose paper. *Pst*I fragments bearing Cγ exons were visualised either using the mouse cDNA probe pγ1Ars (Sims *et al.*, 1982) which contains the whole Cγ1 region or M13/13 (Dunnick *et al.*, 1980) which contains only the CH2 and CH3 domains. The result of the filter hybridisation experiment appears in Figure 4. In clones λHG4.6, 9.6 (γ2), and 4.1 (γ4) the CH1 domain is located on a 1-kb *Pst*I fragment since this fragment hybridises to pγ1Ars (Figure 4A1) but not to M13/13 (Figure 4A2). Clones λHG6.3 and 11.4 lack a hybridising CH1 domain but show hybridisation to M13/13 (CH2 and CH3 probe). This observation places the CH2/CH3 domains at the 5′ end of λHG6.3 and 11.4. The hCH2CH3 domains occur on a 1.7-kb fragment [which hybridises to both pγ1Ars and M13/13 in λHG9.6, 11.4(γ2), and 4.1(γ4)]. γHG6.3 shows an 8-kb fragment hybridising to both probes indicating that this phage does not have the 5′ *Pst*I site within the cloned DNA segment; this result confirms that some part of the hinge to CH3 domains occurs at the 5′ end of the inserted human DNA in this clone. Conversely, λHG4.6 possesses the small *Pst*I fragment containing the CH1 domain but the 1.7-kb fragment containing hinge to CH3 is replaced by the 3-kb fragment. The data described in Figure 1 show that the CH3 domain in λHG4.6 borders the lambda vector DNA, so the γ2 coding segment must occur at the 3′ end of this human insert.

Restriction enzyme mapping of the clone λHG4.1 (which carries the γ4 gene) showed the presence of a 3.1-kb *Eco*RI-*Hind*III fragment, located at one boundary of the insert DNA (i.e., in which the *Eco*RI site joins the human DNA insert to the lambda phage vector), which hybridised to the

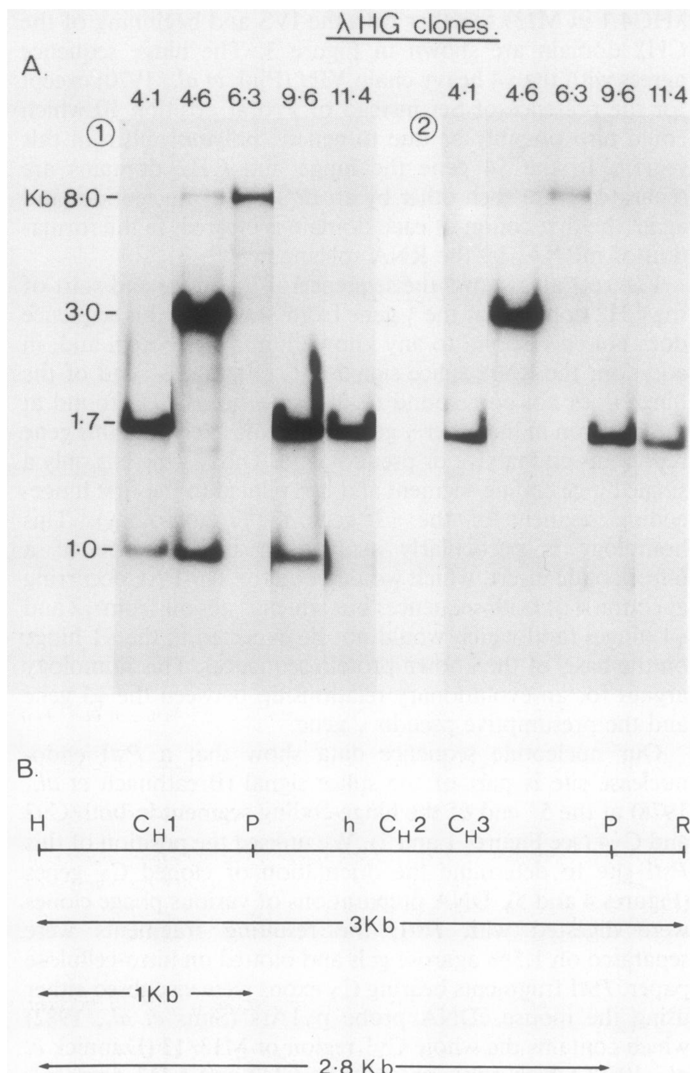

Fig. 4. (A) Orientation of transcription for human Cγ clones. *Pst*I digests of clones λHG4.1, 4.6, 6.3, 9.6, and 11.4 were separated on 1.5% agarose gels and transferred to nitro-cellulose paper. The filters were hybridised to nick-translated probes and washed as described in Materials and methods. The hybridising bands were visualised by autoradiography overnight. The fragments shown for (1) were hybridised to nick-translated pγ1Ars. The fragments in (2) were hybridised to nick-translated M13/13. *Eco*RI fragments of phage lambda DNA and *Hae*III fragments of phage G4 served as mol. wt. markers. **(B)** λHG4.1 DNA was digested with *Hind*III, end-labelled with ^{32}P, and redigested with *Eco*RI. The 3.1-kb *Eco*RI-*Hind*III fragment was isolated, cleaved with *Pst*I, and the resulting fragments analysed on 2% agarose gels. Fragments shown are the sizes of the radioactively labelled bands observed. P = *Pst*I, H = *Hind*III, R = *Eco*RI.

mouse γ1 probe. To determine the γ4 gene orientation within λHG4.1, we mapped the *Pst*I sites in this *Eco*RI-*Hind*III fragment by partial *Pst*I cleavage after ^{32}P-terminal labelling of the *Hind*III site. Figure 4B shows the sizes of the radioactive fragments after *Pst*I digestion plus the deduced arrangement of *Pst*I sites in the 3.1-kb fragments. Two digestion products (2.7 kb and 1 kb) were observed in the experiment showing that a *Pst*I site occurs 1 kb from the *Hind*III site and establishes that the 1.7-kb *Pst*I fragment (containing the hinge to CH2 and CH3) lies closer to the 3′-terminal *Eco*RI site. Thus the γ4 gene in λHG4.1 resides adjacent to the boundary of the human DNA insert and the 1.7-kb *Pst*I fragment carrying the hinge to CH3 domains (i.e., the 3′ end

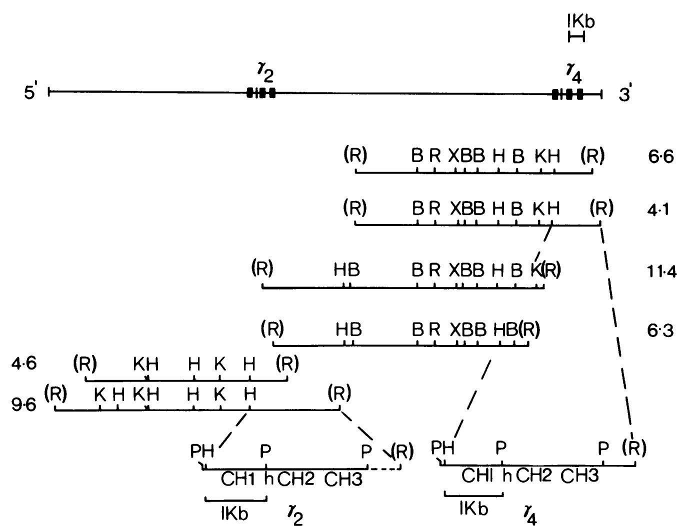

Fig. 5. Physical map of genomic region carrying human Cγ2 and Cγ4 genes. Various restriction enzyme digests of λHG clones were separated on 1% agarose. The gels were stained with ethidium bromide and photographed: afterwards gels were blotted and hybridised to pγ1Ars. Size estimations were made using *Eco*RI cut lambda phage and *Hae*III cut G4 phage. Restriction fragments carrying Cγ exons are mapped for *Pst*I sites (blown scale). Artificial *Eco*RI sites are shown in brackets. The gene order is 5′-Cγ2-Cγ4-3′. R = *Eco*RI, B = *Bam*HI, H = *Hind*III, K = *Kpn*I, X = *Xba*I, P = *Pst*I.

of the gamma coding region) lies nearest to the *Eco*RI site of the lambda vector.

The physical linkage of Cγ2 and Cγ4 genes was determined from a map of restriction endonuclease sites for the enzymes *Eco*RI, *Bam*HI, *Hind*III, *Kpn*I, and *Xba*I in the clones carrying the genes encoding γ2 and γ4 heavy chains (Figure 5). Our map of restriction endonuclease sites in human Cγ genes showed that a cluster of λHG clones carrying the Cγ2 gene (4.6, 9.6, 6.3, 11.4, and 1.4) overlapped a cluster containing the Cγ4 gene (2.1, 4.1, and 6.6). The location of the γ2 coding region within clones containing this gene allowed the ordering of these clones relative to each other as shown in Figure 5 (see previous section). The γ2 and γ4 genes are, therefore, separated from each other by 19 kb of DNA and both genes lie in the same direction of transcription, with γ2 preceding the γ4 gene. It should be noted, however, that the restriction maps of the 5′ ends of the clones λHG4.6 and λHG9.6 differ from each other: we do not know the origin of this difference. It is possible that it represents a polymorphism within the genome from which the phage library was prepared or it may represent the result of deletions occurring during the cloning of those particular gene segments (Marcu *et al.*, 1980).

The overlapping phage clones which carry Cγ2 and Cγ4 genes show that the Cγ4 gene is located 19 kb downstream of Cγ2. We have not yet been able to overlap the other gamma clones which we have isolated. γ2−γ4 is of interest since it is very analogous to the spacing of mouse gamma subclass genes (Shimizu *et al.*, 1981) and indeed shows a very similar separation distance to that previously observed for the human VH genes (Matthyssens and Rabbitts, 1980). This observation has evolutionary implications since it supports the argument that V- and C-regions arose from primordial 'domain' genes by gene duplications (Milstein and Munro, 1973). The gene order 5′-Cγ2-Cγ4-3′ disagrees with the prediction, on the basis of serological data, that Cγ4 is upstream of the Cγ2

gene (Natvig and Kunkel, 1973). This prediction was based on the finding of a hybrid IgG molecule which expressed antigens of the IgG4 subclass on the CH1 and CH2 domains but IgG2 antigens on CH3. In addition, the normal counterpart of this protein has been detected in certain rare Negro sera which appear to result from this rare IgG4-IgG2 hybrid gene in a homozygous state. Although the authors drew the most straightforward conclusion from their serological typing data, it should be pointed out that a part of the Cγ4 gene could have been placed in front of part of the Cγ2 by unequal crossover events such as sister chromatid exchange (Rabbitts *et al.*, 1980) using, for example, homologies in the intervening sequences.

Materials and methods

Cloning procedures

The human foetal liver phage library was kindly provided by T. Maniatis and was constructed by partial *Hae*III-*Alu*I digestion of DNA followed by addition of *Eco*RI linkers and ligation to the lambda phage vector Charon 4A (Blattner *et al.*, 1977). The library was screened as described (Matthyssens and Rabbitts, 1980) by *in situ* hybridisation (Benton and Davis, 1977). The hybridisation was carried out for two days at 65°C using 4 x SSC, 0.1% SDS, 0.4% Ficoll, 0.4% bovine serum albumin (BSA), 0.4% polyvinyl pyrrolidone (PVP), 10 μg/ml *Escherichia coli* DNA (sheared, denatured) (Denhardt, 1966; Jeffreys and Flavell, 1977) followed by four washes in 2 x SSC, 0.1% SDS prior to autoradiography at -70°C with prefogged X-ray film (Laskey and Mills, 1977). Probes were nick-translated (Rigby *et al.*, 1977) to a specific activity of $\sim 5 \times 10^7$ c.p.m./μg. Growth of positively hybridising phage was carried out as described (Bentley and Rabbitts, 1981).

DNA sequencing

Nucleotide sequencing was carried out using M13 single-stranded vectors mp7, mp8, and mp9 (Messing *et al.*, 1981) employing the dideoxy chain termination procedures (Schreier and Cortese, 1979; Sanger *et al.*, 1980). The use of M13mp8 and mp9 for DNA sequencing facilitates the derivation, on both strands, of a nucleotide sequence from a fragment with different restriction sites at each end.

Plasmid cloning

Cloning of the required restriction fragments into pBR322 (Bolivar *et al.*, 1977) or pACYC184 (Chang and Cohen, 1978) was carried out with a 3:1 molar ratio of target to vector DNA. Restriction fragments, used in the ligations, were isolated from low melting temperature agarose gels as described (Sanger *et al.*, 1980).

Restriction enzyme mapping

Restriction enzyme mapping was carried out by digestion with appropriate enzymes followed by fractionation on agarose gels. The gels were stained with ethidium bromide (0.5 μg/ml for 20 min), photographed, and the DNA was transferred to cellulose nitrate filters (Southern, 1975). The filters were hybridised at 65°C overnight with 5×10^5 c.p.m./ml of nick-translated probe (see text) in 6 x SSC, 0.1% SDS, 50 μg sonicated, denatured salmon DNA, 0.2% each of Ficoll, BSA, and PVP. The unhybridised probe was removed from the filters by washing in 1 x SSC, 0.1% SDS, and the hybridisation visualised by autoradiography of the filters at -70°C with prefogged film.

Partial *Pst*I mapping of the *Eco*RI-*Hind*III fragment was carried out as described (Smith and Birnstiel, 1976). λHG4.1 DNA was digested with *Hind*III and restriction fragments were end-labelled with [^{32}P]ATP using the large fragment of DNA polymerase I. The *Hind*III fragments subsequently were cut with *Eco*RI and the 3.1-kb *Eco*RI-*Hind*III fragment which carries the coding region of the Cγ4 gene was isolated on LGT-agarose. This 3.1-kb fragment was then partially cleaved with *Pst*I and the resulting fragments were separated on 2% agarose.

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