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The sequence of a human immunoglobulin episilon heavy chain constant region gene, and evidence for three non-allelic genes

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An immunoglobulin epsilon heavy chain gene was isolated from a DNA library of the human epsilon chain-producing myeloma 266B1, using a J_H gene region probe. The gene was shown to be the one expressed in the myeloma by Southern hybridisation analysis and by comparison of nucleotide sequences with the known amino acid sequence of the epsilon chain made by the myeloma. The gene consists of a variable region segment separated from a constant region segment by a 3.5-kb intervening sequence. The complete sequence of the constant region gene segment shows that this segment is split by intervening sequences into four coding segments corresponding to the four constant region domains of the protein. Using the cloned epsilon constant region gene segment as a probe we obtained evidence, from Southern hybridisation analysis, for three non-allelic epsilon constant region genes. An order on the chromosome for these three genes can be predicted from their pattern of retention in myeloma 266B1 DNA.

Key words: antibody genes/immunoglobulin E/human myeloma/nucleotide sequence

Introduction

Antibodies are composed of heavy (H) and light (L) chains, each of which has a variable (V) and constant (C) region. In man there are five antibody classes, known as IgM, IgD, IgG, IgE, and IgA, which have different biological activities and are structurally distinguished by their C_H regions ($C\mu$, $C\delta$, $C\gamma$, $C\epsilon$, and $C\alpha$, respectively). Protein sequencing has also revealed four IgG and two IgA subclasses, which can show further differences in biological activity.

The genes which encode these proteins in humans consist of multiple V_H gene segments (Matthyssens and Rabbitts, 1980) linked to a small number of C_H gene segments on chromosome 14 (Croce *et al.*, 1979; Hobart *et al.*, 1981). B-lymphocyte differentiation involves sequential usage of C_H genes. The $C\mu$ gene is the first to be expressed. The active human μ gene is formed by integration of V_H and D gene segments with a J_H segment which is located upstream of the $C\mu$ gene (Matthyssens and Rabbitts, 1980; Siebenlist *et al.*, 1981; Rabbitts *et al.*, 1981; Ravetch *et al.*, 1981). Studies in mouse and man have revealed something of the way in which subsequent C_H gene switching occurs. $C\mu$ and $C\delta$ genes are co-expressed by differential processing of a large RNA transcript which includes the V_H segment and both $C\mu$ and $C\delta$ (Maki *et al.*, 1981; Rabbitts *et al.*, 1981; Knapp *et al.*, 1982). The class switch in antibody secreting cells from $C\mu$ to $C\gamma$, $C\epsilon$, or $C\alpha$, however, involves deletion of DNA (Honjo and Kataoka, 1978; Coleclough *et al.*, 1980; Cory and Adams, 1980; Rabbitts *et al.*, 1980a) so that the V_H segment is transcribed with the next C_H gene. This deletion involves recombination between S segments located upstream of each C_H gene except $C\delta$ (Davis *et al.*, 1980; Kataoka *et al.*, 1980; Rabbitts *et al.*, 1981). These S segments in mouse and man were found to contain short tandem repeated sequences (Dunnick *et al.*, 1980; Rabbitts *et al.*, 1981; Obata *et al.*, 1981) which are probably involved in homologous recombination, perhaps by sister chromatid exchange (Rabbitts *et al.*, 1980b).

Human IgE is medically important as the mediator of allergic reactions. However, only one $C\epsilon$ protein sequence is available (Bennich and von Bahr-Lindström, 1974) and there is no information on possible IgE subclasses. Furthermore, nothing is known about human ϵ genes. Portions of sequence near the end of the mouse ϵ gene (Nishida *et al.*, 1981) and near the end of the rat cDNA (Hellman *et al.*, 1982) have been published but no complete $C\epsilon$ gene sequence is available for any species.

We describe here the isolation of a human ϵ gene from an ϵ-producing myeloma. The cloning strategy was to construct a DNA library from the myeloma and detect clones containing the active ϵ gene using a J_H gene region probe. This approach relies on the retention in the active ϵ gene of sequences which, before H chain switching, were between the J_H segments and the $C\mu$ gene. We have shown that the cloned ϵ gene is the one expressed in the myeloma and we present the complete sequence of the $C\epsilon$ gene segment. Using the $C\epsilon$ gene segment as a hybridisation probe, we have shown that there are at least three non-allelic $C\epsilon$-like genes in the human genome.

Results and Discussion

Isolation of the expressed ϵ gene from a human myeloma cell line

In order to isolate the expressed ϵ gene from the ϵ-producing human myeloma 266B1 (Nilsson *et al.*, 1970) *Sau*3AI partial digestion fragments of 266B1 DNA were cloned in the phage vector λ1059 (Karn *et al.*, 1980). The resulting library was screened with mixed probes C76R51A and C76R51B which together contain a 3.8-kb sequence from the J_H gene region of human foetal liver DNA (Figure 1 and Materials and methods). A positive clone, designated $\lambda\epsilon$1.2, was isolated from the library and studied by restriction mapping, hybridisation, and sequencing.

Figure 2 shows a restriction map of $\lambda\epsilon$1.2. V_H and $C\epsilon$ gene segments were initially located within the clone by Southern hybridisation: the probe for V_H was a V_HIII gene clone (V_H52-1) (Matthyssens and Rabbitts, 1980) while the probe for $C\epsilon$ was short cDNA made on total poly(A)$^+$ RNA from 266B1 myeloma cells. The V and C segments were subsequently characterised by nucleotide sequencing. Figure 2 includes the nucleotide sequence corresponding to the start of the V_H segment (obtained by sequencing from a *Bam*HI site located within the leader segment) extending for 21 codons into framework region one. The derived amino acid sequence corresponds exactly to that of the V_HI chain expressed by

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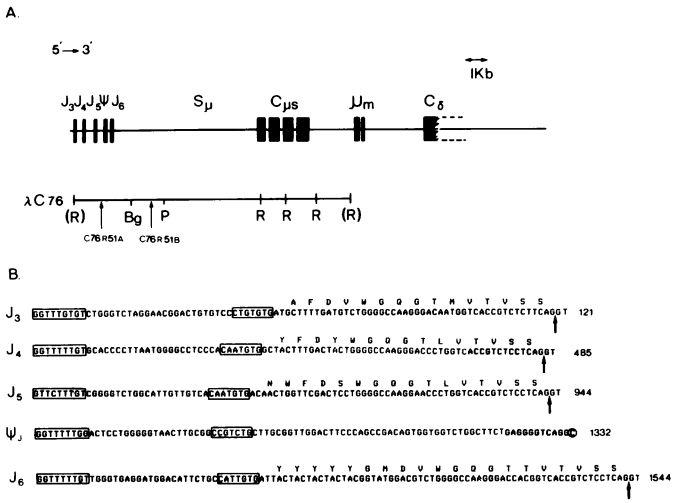


Fig. 1. Relationship of J_H probe to arrangement of Cμ and Cδ genes in human DNA. **A.** R = *Eco*RI, Bg = *Bgl*II, P = *Pst*I, Sμ = switch region of μ gene, ψ = position of ψ J_H segment. The only *Bgl*II and *Pst*I sites shown are those at the ends of the C76R51A and C76R51B sequences. **B.** Sequences shown are those encoding the J_H segments plus the homology boxes similar to those adjacent to the 3′ end of the human V_H genes. The arrows represent RNA splice sites, the circle represents an altered splice site (GGC) of the ψJ. The derived amino acid sequence of the J segments is shown in the single letter code.

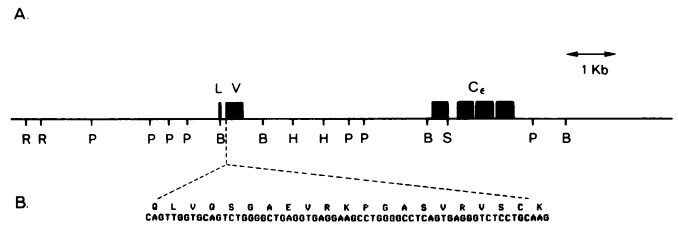


Fig. 2. The expressed ε gene of myeloma 266B1. **A.** Restriction map of the λε1.2 insert. R = *Eco*RI, B = *Bam*HI, P = *Pst*I, H = *Hind*III, S = *Sal*I. Protein coding regions are shown by raised boxes. The L (leader), V, and Cε regions were identified by nucleotide sequencing. **B.** Nucleotide sequence of the 5′ end of the V_H segment. The deduced amino acid sequence is shown in the single letter code above the nucleotide sequence.

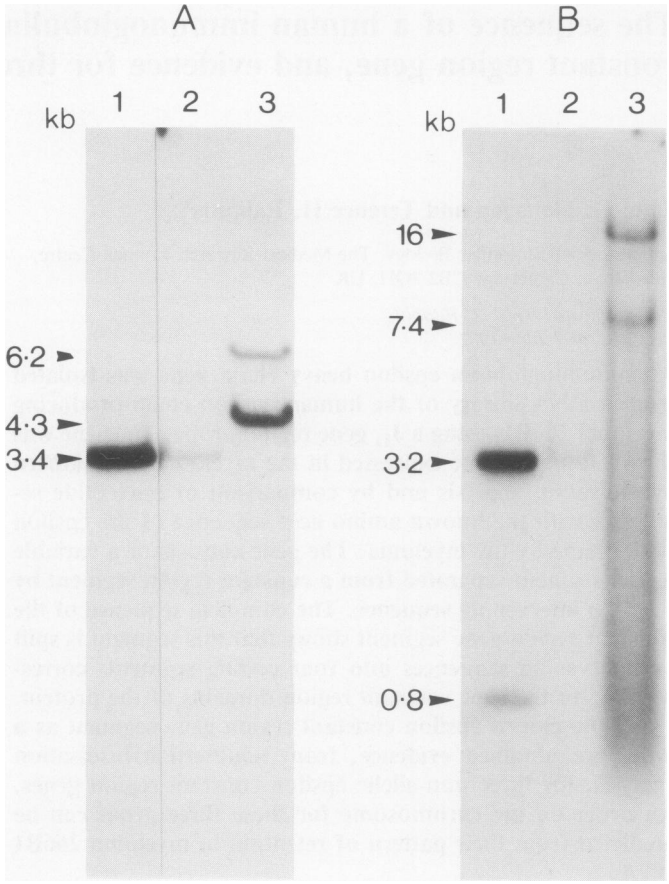


Fig. 3. Southern blot hybridisation of J_H gene region probes to λε1.2, myeloma 266B1 DNA, and placental DNA. The DNAs were digested with *Pst*I (**A**) or *Bam*HI (**B**). In **A** the probe was C76R51B, while in **B** mixed C76R51A and C76R51B were used. (**1**) λε1.2, (**2**) 266B1 myeloma DNA, (**3**) placental DNA. On filter **A, lane 1** was exposed for a shorter period than the others in order to make band intensities more similar. The two placental DNAs used in this experiment were from different sources.

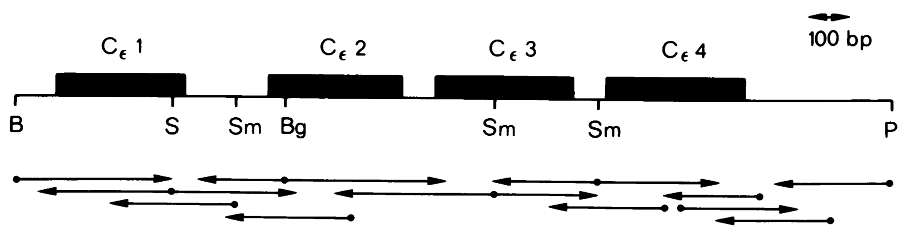
266B1 cells (Bennich and von Bahr-Linsdström, 1974). This correspondence strongly suggests that λε1.2 contains the ε gene which is expressed in myeloma 266B1. This conclusion was confirmed by Southern filter hybridisation comparing λε1.2, 266B1 myeloma DNA, and human placental DNA (Figure 3). The DNAs were digested with either *Pst*I (filter A) or *Bam*HI (filter B) and hybridised with the J_H gene region probes C76R51A and C76R51B. Both digests of 266B1 DNA (slots A2 and B2) showed single hybridisation bands (3.4 kb *Pst*I and 3.2 kb *Bam*HI) different from any bands in placental DNA (slots A3 and B3). This implies that 266B1 myeloma DNA contains only a single H chain gene locus, and the bands presumably represent the rearranged, active ε chain gene. The bands from 266B1 DNA coincide with bands from λε1.2 (shown in slots A1 and B1) confirming that the λε1.2 clone contains an accurate copy of the active ε gene. An additional hybridising 0.8-kb *Bam*HI fragment from λε1.2 (slot B1) is visible but this fragment was too small to give a detectable band in the digest of 266B1 genomic DNA. It is interesting that two different placental DNAs showed two bands of hybridisation with the J_H region probes despite the absence of *Bam*HI and *Pst*I sites from the probe sequences. This implies a high degree of polymorphism within the J_H area in humans as previously described in mice (Marcu *et al.*, 1980; Forster *et al.*, 1980).

λε1.2 thus represents the active ε gene from 266B1 DNA containing a V_H gene segment and a Cε gene segment (described below) separated by an intervening sequence of ~3.5 kb. Comparison of λε1.2 with the human foetal liver J_H region in λC76 by restriction mapping and Southern hybridisation indicates that the point at which H chain switch recombination occurred to produce the active ε gene was <1.5 kb upstream of the Cε segment.

Complete nucleotide sequence of a human Cε gene

Figure 4 shows the sequencing strategy and the complete nucleotide sequence of the Cε gene segment in λε1.2. The amino acid sequence deduced from the nucleotide sequence matches the published sequence for the ε chain produced by 266B1 cells (Bennich and von Bahr-Lindström, 1974) except for 15 discrepancies, which probably result from protein sequencing errors. The terminal codon of Cε4 codes for lysine (before a TGA chain termination codon). The ε protein made by the 266B1 myeloma was found to possess a heterogeneous carboxy terminus (equimolar yields of C-terminal Gly-Lys and Gly were detected) and it was suggested that loss of Lys from some molecules resulted from carboxypeptidase activity

A.



B.

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                                                                                                       A S T Q S P S V
GGATCCCTGCCACGGGGTCCCCAGCTCCCCCATCCAGGCCCCCCAGGCTGATGGGCGCTGGCCTGAGGCTGGCACTGACTAGGTTCTGTCCTCACAGCCTCCACACAGAGCCCATCCGTC   120

F P L T R [C] (A) K N I P S N A T S V T L G (C) L A T G Y F P E P V K V T W D T G S L
TTCCCCTTGACCCGCTGCTGCAAAAACATTCCCTCCAATGCCACCTCCGTGACTCTGGGCTGCCTGGCCACGGGCTACTTCCCGGAGCCGGTGATGGTGACCTGGGACACAGGCTCCCTC   240

        - -
N G T T M T L P A T T L T L S G H Y A T I S L L T V S G A W A K Q M F T (C) R V A
AACGGGACAACTATGACCTTACCAGCCACCACCCTCACGCTCTCTGGTCACTATGCCACCATCAGCTTGCTGACCGTCTCGGGTGCGTGGGCCAAGCAGATGTTCACCTGCCGTGTGGCA   360

            - N V K -
H T P S S T D W V D N K T F S
CACACTCCATCGTCCACAGACTGGGTCGACAACAAAACCTTCAGCGGTAAGAGAGGGCCAAGCTCAGAGACCACAGTTCCCAGGAGTGCCAGGCTGAGGGCTGGCAGAGTGGGCAGGGGT   480

TGAGGGGGTGGGTGGGCTCAAACGTGGGAACACCCAGCATGCCTGGGGACCCGGGCCAGGACGTGGGGGCAAGAGGAGGGCACACAGAGCTCAGAGAGGCCAACAACCCTCATGACCACC   600

                                        L                 -
             V (A) S R D F T P P T V K I L Q S S [C] D G G G H F P P T I Q L L (C) L V S G
AGCTCTCCCCCAGTCTGCTCCAGGGACTTCACCCCGCCCACCGTGAAGATCTTACAGTCGTCCTGCGACGGCGGCGGGCACTTCCCCCCGACCATCCAGCTCCTGTGCCTCGTCTCTGGG   720

                                                E S                   E
Y T P G T I N I T W L E D G Q V M D V D L S T A S T T Q E G E L A S T Q S E L T
TACACCCCAGGGACTATCAACATCACCTGGCTGGAGGACGGGCAGGTCATGGACGTGGACTTGTCCACCGCCTCTACCACGCAGGAGGGTGAGCTGGCCTCCACACAAAGCGAGCTCACC   840

L S Q K H W L S D R T Y T (C) Q V T Y Q G H T F E D S T K K [C] A
CTCAGCCAGAAGCACTGGCTGTCAGACCGCACCTACACCTGCCAGGTCACCTATCAAGGTCACACCTTTGAGGACAGCACCAAGAAGTGTGCAGGTACGTTCCCACCTGCCCTGGTGGCC   960

                                                    D
                                                  D S N P R G V S A Y L S R P S P F D L F I
GCCACGGAGGCCAGAGAAGAGGGGCGGGTGGGCCTCACACAGCCCTCCGGTGTACCACAGATTCCAACCCGAGAGGGGTGAGCGCCTACCTAAGCCGGCCCAGCCCGTTCGACCTGTTCA   1080

R K S P T I T (C) L V V D L A P S K G T V N L T W S R A S G K P V N H S T R K E E
TCCGCAAGTCGCCCACGATCACCTGTCTGGTGGTGGACCTGGCACCCAGCAAGGGGACCGTGAACCTGACCTGGTCCCGGGCCAGTGGGAAGCCTGTGAACCACTCCACCAGAAAGGAGG   1200

K Q R N G T L T V T S T L P V G T R D W I E G E T Y Q (C) R V T H P H L P R A L M
AGAAGCAGCGCAATGGCACGTTAACCGTCACGTCCACCCTGCCGGTGGGCACCCGAGACTGGATCGAGGGGGAGACCTACCAGTGCAGGGTGACCCACCCCCACCTGCCCAGGGCCCTCA   1320

R S T T K T S                                                                                      G P R A A
TGCGGTCCACGACCAAGACCAGCGGTGAGCCATGGGCAGGCCGGGGTCGTGGGGGAAGGGAGGGAGCGAGTGAGCGGGGCCCGGGCTGACCCCACGTCTGGCCACAGGCCCGCGTGCTGC   1440

P E V Y A F A T P E W P G S R D K R T L A (C) L I Q N F M P E D I S V Q W L H N E
CCCGGAAGTCTATGCGTTTGCGACGCCGGAGTGGCCGGGGAGCCGGGACAAGCGCACCCTCGCCTGCCTGATCCAGAACTTCATGCCTGAGGACATCTCGGTGCAGTGGCTGCACAACGA   1560

                                                                                Q E
V Q L P D A R H S T T Q P R K T K G S G F F V F S R L E V T R A E W E Q K D E F
GGTGCAGCTCCCGGACGCCCGGCACAGCACGACGCAGCCCCGCAAGACCAAGGGCTCCGGCTTCTTCGTCTTCAGCCGCCTGGAGGTGACCAGGGCCGAATGGGAGCAGAAAGATGAGTT   1680

I (C) R A V H E A A S P S Q T V Q R A V S V N P G K *
CATCTGCCGTGCAGTCCATGAGGCAGCGAGCCCCTCACAGACCGTCCAGCGAGCGGTGTCTGTAAATCCCGGTAAATGACGTACTCCTGCCTCCCTCCCTCCCAGGGCTCCATCCAGCTG   1800

TGCAGTGGGGAGGACTGGCCAGACCTTCTGTCCACTGTTGCAATGACCCCAGGAAGCTACCCCCAATAAACTGTGCCTGCTCAGAGCCCCAGTACACCCATTCTTGGGAGCGGGCAGGGC   1920
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Fig. 4. Nucleotide sequence of the C_ϵ gene segment from $\lambda\epsilon 1.2$. **A.** The sequencing strategy. The C_ϵ coding segments are shown by raised boxes. Fragments for sequencing were generated using the restriction enzymes shown, or by sonication (Fuhrman *et al.*, 1981). B = *Bam*HI, P = *PstI*, *S* = *Sal*I, Bg = *Bgl*II, Sm = *Sma*I. **B.** The C_ϵ nucleotide sequence. The deduced amino acid sequence for the coding regions is shown in the single letter code above the nucleotide sequence. The letters above the derived amino acid sequence indicate discrepancies with the previously published protein sequence and dashes indicate residues absent from that sequence. The characteristic recognition sequence for polyadenylation is underlined and the RNA splice sites are arrowed. Cysteine residues involved in conserved intradomain disulphide bonds are circled, those involved in an additional intrachain bond are enclosed by triangles, and those involved in interchain bonds are enclosed by squares (the first of these joins to L chain and the other two to H chain). Glycosylated asparagine residues are underlined.

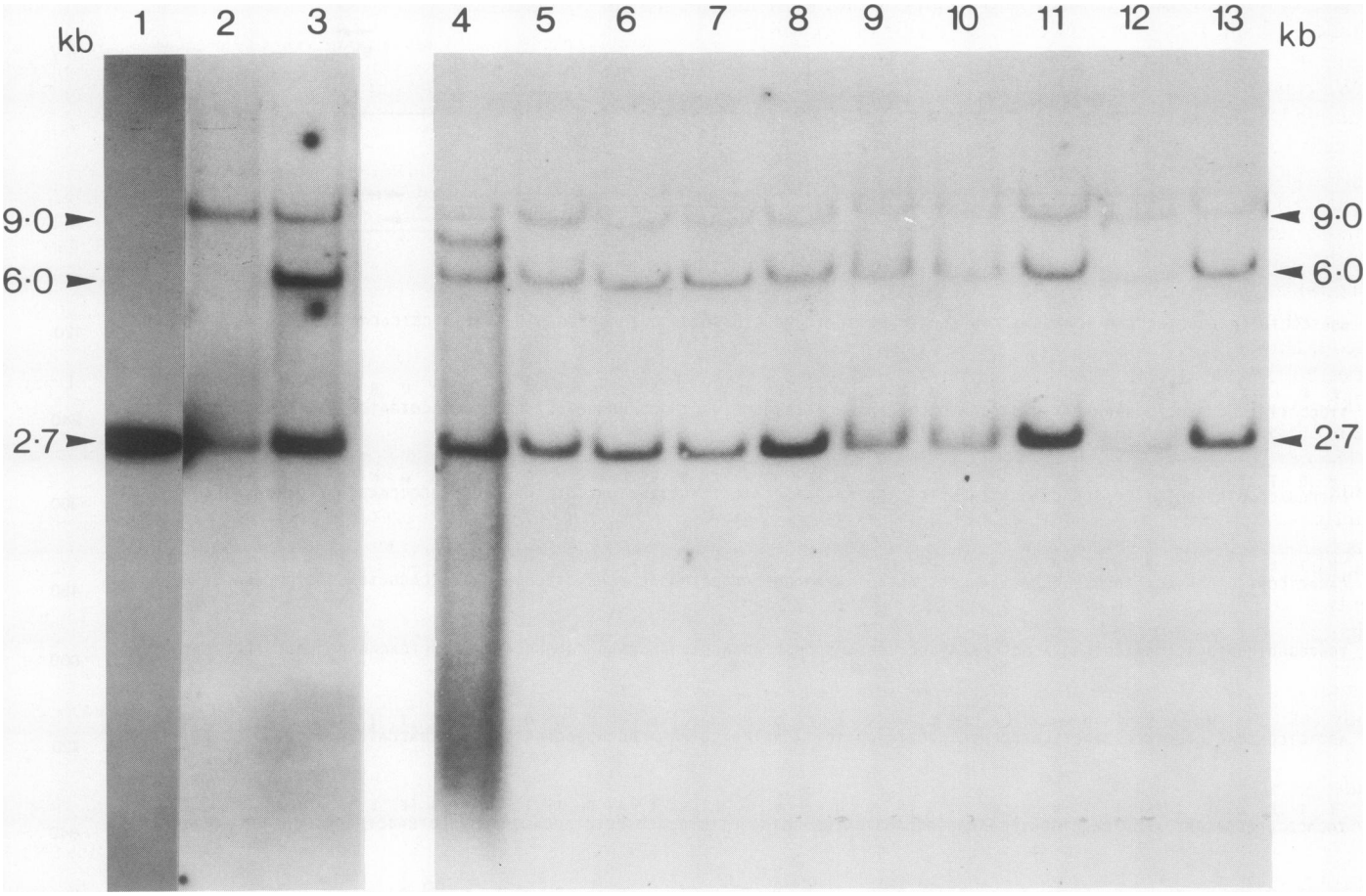

Fig. 5. Hybridisation of a Cε probe to λε1.2, 266B1 myeloma DNA, and genomic DNA from non-IgE-producing cells of 10 unrelated individuals. All the DNAs were digested with *Bam*HI. The probe was nick-translated ε1.2BP25. The sources of the human DNAs were as follows: **(1)** λε1.2 phage, **(2)** 266B1 myeloma, **(3)**, **(4)**, **(6)**, and **(7)** placentas – **lanes 3** and **7** are from the same source, **(5)** liver, **(8)** spleen from an Italian thalassaemic patient, **(9)** Daudi, an IgM-producing line from an African patient with Burkitt's lymphoma (Klein *et al.*, 1968), **(10)** Raji, an IgM-producing line also from a Burkitt's lymphoma patient (Pulverlaft, 1965), **(11)** Molt 4 cells from an acute lymphoblastic leukaemia patient (Minowada *et al.*, 1972), **(12)** HMy2, an IgG-producing lymphoblastoid cell line (Sikora *et al.*, 1982), **(13)** HeLa cells. **Lane 1** was exposed for a shorter period than the others in order to make band intensities more similar.

(Bennich *et al.*, 1973). The Cε gene is split into four coding segments corresponding closely to the four domains of the C_H region of the protein. We found no separate hinge-coding segment. The human Cγ genes were found to possess small separate hinge coding segments (Ellison and Hood, 1982; Krawinkel and Rabbitts, 1982) so the absence of a separate hinge-coding segment from human Cε, like Cμ (Ravetch *et al.*, 1980; Takahashi *et al.*, 1980; Rabbitts *et al.*, 1981), implies that the hinge domain arose within the γ genes after divergence from Cμ and Cε genes. The splice sites at the intervening sequence boundaries are homologous to the consensus sequences deduced for other genes (Mount, 1982) and 106 bases from the Cε4 termination codon is the sequence A-A-T-A-A-A which is characteristic of polyadenylation sites, thus defining the end of the gene for the secreted ε chain.

Evidence for three non-allelic Cε genes in human DNA

The availability of a cloned Cε gene enabled us to perform Southern blotting experiments to study the number of Cε genes in human DNA. For use as a Cε probe, the 2.1-kb *Bam*HI to *Pst*I fragment containing the Cε gene of λε1.2 was subcloned into M13mp701. Figure 5 shows Southern blots in which this subclone, ε1.2BP25, was used to probe *Bam*HI digests of λε1.2, 266B1 myeloma DNA, and DNA of non-IgE-producing cells from 10 unrelated individuals. 266B1 myeloma DNA showed two hybridising bands at 9.0 kb and 2.7 kb (slot 2). The 2.7-kb band coincides with the band detected in λε1.2 (slot 1), and we have shown by sequencing that this 2.7-kb fragment of λε1.2 contains the entire Cε gene expressed by 266B1 cells. As discussed earlier, the 266B1 myeloma cell line seems to possess only one chromosome carrying H chain genes, so the results indicate that two non-allelic Cε-like genes exist in 266B1 DNA.

When the Cε probe was hybridised with 10 other human DNAs which do not express IgE, we found evidence for a third Cε gene. In addition to the 9.0-kb and 2.7-kb *Bam*HI fragments found in the 266B1 DNA, we detected a further 6.0-kb band in the other human DNAs studied, including DNA from cells producing IgM (Raji and Daudi) or IgG (HMy2) (Figure 5). The presence of the three hybridisation components in these randomly selected human DNAs indicates that they do not segregate in a Mendelian fashion and argues strongly for the existence of three non-allelic Cε-like genes. One of the placental DNA samples used in our analysis showed, in addition to the three hybridisation bands discuss-

ed above, a fourth *Bam*HI restriction fragment (slot 4). This additional band may represent either an allelic or non-allelic segment.

We can predict an order for the three Cε-like sequences in genomic DNA. The H chain class switch in antibody-producing cells results in deletion of all C_H genes between the active V_H segment and the newly-active C_H segment. Therefore, the absence of the 6.0-kb hybridising fragment from 266B1 DNA could be explained in terms of deletion of this fragment during the activation of the gene on the 2.7-kb fragment. Thus the 6.0-kb fragment is probably upstream of the 2.7-kb fragment, while the 9.0-kb fragment is probably downstream since it is retained in the ε myeloma DNA. The order of the genes on the chromosome is thus likely to be 5′-Cμ-6kbCε-2.7kbCε-9kbCε-3′ although it is, of course, possible that they are not tandemly associated.

The data presented above indicate that the human genome contains at least three non-allelic Cε-like genes. This finding contrasts with studies on the mouse where only one Cε gene was found and no further Cε-like sequences were detected by hybridisation (Nishida *et al.*, 1981). We do not know whether the two Cε-like genes that we detected by hybridisation only (Figure 5) are potentially active genes or inactive pseudogenes. Cloning and characterisation of the relevant areas of human DNA should help us resolve this issue, and allow us to study the gene organisation in more detail.

Materials and methods

Construction and screening of a phage λ library

DNA from the human IgE-producing myeloma cell line 266B1 was partially digested with *Sau*3A1 and fractionated on a sucrose gradient as previously described (Dunnick *et al.*, 1980) to obtain fragments of ~15–20 kb. These were ligated to *Bam*HI-cut λ1059 (Karn *et al.*, 1980) and packaged *in vitro*. The resulting library of ~2 x 10^6 recombinants was then amplified and library screening was performed using ~5 x 10^6 p.f.u. of the amplified library. Cellulose nitrate filter replicas of the phage plates were made (Benton and Davis, 1977) and the filters were hybridised to nick-translated probe (see below) in 4 x SSC at 65°C. After hybridisation for 2 days with 3 x 10^5 c.p.m./ml of probe, the filters were washed in 2 x SSC, 0.1% SDS for 4 h, then autoradiographed using an intensifying screen (Laskey and Mills, 1977). The J_H gene region probe came from a 3.8-kb *Eco*RI to *Pst*I fragment from the 5′ end of phage λC76 (Rabbitts *et al.*, 1981) which is derived from human foetal liver DNA. The 5′ end of this fragment (Figure 1) was shown by nucleotide sequencing (as described below) to contain four J_H segments and a pseudo J_H which are similar to J3 to J6 plus the pseudo J_H previously described (Ravetch *et al.*, 1981). For use as a probe, this *Eco*RI to *Pst*I fragment was subcloned in two pieces in M13mp7: C76R51A (2.5 kb, *Eco*RI to *Bgl*II) and C76R51B (1.3 kb, *Bgl*II to *Pst*I). Figure 1 depicts the relationship of these J_H probes, in the λC76 clone, to the organisation of human genomic DNA in the area of the Cμ and Cδ genes.

Southern filter hybridisation

Probe DNA was labelled to a specific activity of ~2 x 10^8 c.p.m./μg by nick-translation (Rigby *et al.*, 1977). Transfer of DNA to nitro-cellulose filters and hybridisation were carried out as described (Denhardt, 1966; Southern 1975; Jeffreys and Flavell, 1977). The salt conditions for hybridisation were 6 x SSC at 65°C, and post-hybridisation washes were carried out in 1 x SSC at 65°C. For hybridisations requiring a cDNA probe derived from ε mRNA, RNA was prepared as described (Auffrey and Rougeon, 1980) and the poly(A)$^+$ fraction isolated on oligo(dT) cellulose. cDNA synthesis was performed as in Rabbitts *et al.* (1977) using pT_{10} as the oligonucleotide primer.

Nucleotide sequencing

The dideoxy chain termination procedure was used in all experiments (Sanger *et al.*, 1980) utilising fragments cloned in M13mp7 (Messing *et al.*, 1981) or M13mp8 and M13mp9.

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References

Auffrey,C., and Rougeon,F. (1980) *Eur. J. Biochem.*, **107**, 303-314.
Bennich,H., Milstein,C., and Secher,D.S. (1973) *FEBS Lett.*, **33**, 49-53.
Bennich,H., and von Bahr-Lindström,H. (1974) in Brent.L., and Holborow, J. (eds.), *Progress in Immunology I*, North-Holland Publishing Company, pp. 49-58.
Benton,W.D., and Davis,R.W. (1977) *Science (Wash.)*, **196**, 180-182.
Coleclough,C., Cooper,D., and Perry,R.P. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 1422-1426.
Cory,S., and Adams,J.M. (1980) *Cell*, **19**, 37-51.
Croce,C.M., Shander,M., Martinis,J., Cicurel,L., D'Amona,G.G., Dolby, T.W., and Koprowski,H. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 3416-3419.
Davis, M.M., Calame,K., Early,P.W., Livant,D.L., Joho,R., Weissman,I.L. and Hood,L. (1980) *Nature*, **283**, 733-739.
Denhardt,D.T. (1966) *Biochem. Biophys. Res. Commun.*, **23**, 641-646.
Dunnick,W., Rabbitts,T.H., and Milstein,C. (1980) *Nature*, **286**, 669-675.
Ellison,J., and Hood,L. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 1984-1988.
Forster,A., Hobart,M., Hengartner,H., and Rabbitts,T.H. (1980) *Nature*, **286**, 897-899.
Fuhrman,S.A., Deininger,P.L., LaPorte,P., Friedmann,T., and Geiduschek, E.P. (1981) *Nucleic Acids Res.*, **9**, 6439-6456.
Hellman,L., Pettersson,U., and Bennich,H. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 1264-1268.
Hobart,M.J., Rabbitts,T.H., Goodfellow,P.N., Solomon,E., Chambers,S., Spurr,N., and Povey,S. (1981) *Ann. Hum. Genet.*, **45**, 331-335.
Honjo,T., and Kataoka,T. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 2140-2144.
Jeffreys,A.J., and Flavell,R.A. (1977) *Cell*, **12**, 429-439.
Karn,J., Brenner,S., Barnett,L., and Cesarini,G. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 5172-5176.
Kataoka,T., Kawakami,T., Takahashi,N., and Honjo,T. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 919-923.
Klein,E., Klein,G., Nadkarni,J.S., Nadkarni,J.J., Wigzell,H., and Clifford, P. (1968) *Cancer Res.*, **28**, 1300-1310.
Knapp,M.R., Liu,C.P., Newell,N., Ward,R.B., Tucker,P.W., Strober,S., and Blattner,F. (1982) *Proc. Natl. Acad. Sci.*, **79**, 2996-3000.
Krawinkel,U., and Rabbitts,T.H. (1982) *EMBO J.*, **1**, 403-407.
Laskey,R.A., and Mills,A.D. (1977) *FEBS Lett.*, **82**, 314-316.
Maki,R., Roeder,W., Traunecker,A., Sidman,C., Wabl,M., Raschke,W., and Tonegawa,S. (1981) *Cell*, **24**, 353-365.
Marcu,K.B., Banerji,J., Penncavage,N.A., Lang,R., and Arnheim,N. (1980) *Cell*, **22**, 187-196.
Matthyssens,G., and Rabbitts,T.H. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 6561-6565.
Messing,J., Crea,R., and Seeburg,P.H. (1981) *Nucleic Acids Res.*, **9**, 309-321.
Minowada,J., Ohnuma,T., and Moore,G.E. (1972) *J. Natl. Cancer Inst.*, **49**, 891-895.
Mount,S.M. (1982) *Nucleic Acids Res.*, **10**, 459-472.
Nilsson,K., Bennich,H., Johansson,S.G.O., and Pontéu,J. (1970) *Clin. Exp. Immunol.*, **7**, 477-489.
Nishida,Y., Kataoka,T., Ishida,N., Nakai,S., Kishimoto,T., Böttcher,I., and Honjo,T. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 1581-1585.
Obata,M., Kataoka,T., Nakai,S., Yamagishi,H., Takahashi,N., Yamawaki-Kataoka,Y., Nikaido,T., Shimizu,A., and Honjo,T. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 2437-2441.
Pulverlaft,R.J.V. (1965) *J. Clin. Pathol.*, **18**, 261-273.
Rabbitts,T.H., Forster,A., Dunnick,W., and Bentley,D.L. (1980a) *Nature*, **283**, 351-356.
Rabbitts,T.H., Hamlyn,P.H., Matthyssens,G., and Roe,B.A. (1980b) *Can. J. Biochem.*, **58**, 176-187.
Rabbitts,T.H., Forster,A., and Milstein,C.P. (1981) *Nucleic Acids Res.*, **9**, 4509-4524.
Rabbitts,T.H., Forster,A., Smith,M., and Gilham,S. (1977) *Eur. J. Immunol.*, **7**, 43-48.
Ravetch,J.V., Kirsch,I.R., and Leder,P. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 6734-6738.

Ravetch,J.V., Siebenlist,U., Korsmeyer,S., Waldmann,T., and Leder,P. (1981) *Cell,* **27**, 583-591.
Rigby,P.W.J., Dieckmann,M., Rhodes,C., and Berg,P. (1977) *J. Mol. Biol.,* **133**, 237-251.
Sanger,F., Coulson,A.R., Barrell,B.G., Smith,A.J.H., and Roe,B.A. (1980) *J. Mol. Biol.,* **143**, 161-178.
Siebenlist,U., Ravetch,J.V., Korsmeyer,S., Waldmann,T., and Leder,P. (1981) *Nature,* **294**, 631-635.
Sikora,K., Alderson,T., Phillips,J., and Watson,J.V. (1982) *Lancet,* **i**, 11-14.
Southern,E.M. (1975) *J. Mol. Biol.,* **98**, 503-517.
Takahashi,N., Nakai,S., and Honjo,T. (1980) *Nucleic Acids Res.,* **8**, 5983-5991.