Structure of the Genes That Rearrange in Development

Mapping of Heavy Chain Genes for Mouse Immunoglobulins M and D

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Summary. A single DNA fragment containing both μ and δ immunoglobulin heavy chain genes has been cloned from normal BALB/c mouse liver DNA with a new λ phage vector Charon 28. The physical distance between the membrane terminal exon of μ and the first domain of δ is 2466 base pairs, with δ on the 3′ side of μ. A single transcript could contain a variable region and both μ and δ constant regions. The dual expression of immunoglobulins M and D on spleen B cells may be due to alternate splicing of this transcript.

In the normal mouse, B lymphocytes are constantly being produced from stem cells located in the bone marrow (*1*). Immature bone marrow cells bearing surface antibody of the immunoglobulin M (IgM) class migrate to the spleen and lymph nodes and divide to give more mature cells bearing both IgM and IgD (*2*, *3*). On a given cell these share a common idiotype (*4*), which indicates that the two antibody molecules have the same or at least a very similar sequence in the variable (V) region. Double producer cells constitute a majority of splenic B lymphocytes (*2*), which after specific antigenic stimulation differentiate further into plasma cells capable of secreting antibody molecules. At some point in this differentiation, some B cells bearing both IgM and IgD can undergo a "class switch" leading to expression of one of the other antibody classes (IgG, IgA, or IgE). In mouse serum, IgM is plentiful but IgD is present at an extremely low level (*5*).

Both the switch to secretion and the class switch are achieved by nucleic acid rearrangements that lead to changes in the protein structure of the antibodies. There are several reports (*6*) indicating that the secreted IgM and the membrane IgM differ at their carboxyl termini. Two reports (*7*) described a molecular mechanism that could explain the structural changes accompanying secretion for IgM. They found two forms of messenger RNA (mRNA) that code for differing carboxyl terminal sequences on the μ heavy chain. One form coded for the known sequence of the secreted IgM protein. The other coded for the same sequence, except that 20 amino acids at the carboxyl terminal were replaced by a new stretch of 41 amino acids having an extremely hydrophobic core of 26 residues; they proposed that this region could serve as a transmembrane anchor for the surface form of IgM. The gene segments coding for these alternative carboxyl terminals were found in the DNA. The coding sequence for the terminus of the secreted form was contiguous to the last constant (C) region domain ($C\mu4$) of the heavy chain, whereas the terminus for the membrane form was 1.85 kilobase pairs (kbp) on the 3′ side of $C\mu4$. The authors proposed that expression of the two forms of the μ chain is controlled through alternate splicing of mRNA precursor of differing lengths, governed by the choice of RNA termination-polyadenylation sites.

The class switch is a more drastic change, involving complete replacement of one heavy chain C region (C_H) with another of different class. The mouse has at least eight C_H genes (μ, δ, γ_3, γ_1, γ_{2b}, γ_{2a}, α, and ϵ) that code, respectively, for the C_H regions of the eight classes (IgM, IgD, IgG3, IgG1, IgG2b, IgG2a, IgA, and IgE). Thus it seems difficult to invoke RNA splicing for such a switch since an RNA precursor of many hundred kilobases would be needed. Honjo and Kataoka (*8*) have proposed a DNA deletion model for the class switch in which the C_H genes in genomic DNA are arranged in tandem on the 3′ side of the heavy chain variable region (V_H) genes. Expression of Ig class in this model is controlled by a somatic deletion of DNA so as to eliminate unexpressed intervening C_H genes. In this way, any V_H gene could ultimately be associated with any of the C_H genes in different cells without the need for splicing out huge intervening sequences. Honjo proposed that the $C\mu$ gene is first in the cluster and is followed by the four $C\gamma$ genes and the $C\alpha$ gene.

Results of recent studies (*9*, *10*), in which the Southern hybridization meth-

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od was used to probe the DNA of plasmacytomas, which are tumors of antibody-secreting cells, strongly support the deletion model and the gene order proposed by Honjo. Cloning of DNA from plasmacytomas has also revealed rearrangements that would be consistent with a deletion model for the class switch (*10*). Of course DNA cloning studies cannot easily discriminate between deletional mechanisms and other types of rearrangement.

The spacing and order of genes for C_H regions are just beginning to be determined by "chromosome walking experiments" in which a series of overlapping molecular clones that span the interval between C_H genes are isolated. Honjo (*11*) has evidence that $C\gamma_{2b}$ and $C\gamma_{2a}$ are about 20 kbp apart and Maki *et al.* (*12*) find a similar distance for $C\gamma_1$ to $C\gamma_{2b}$. We find that the J_H cluster is 8 kbp from $C\mu$ and that $C\mu$ is probably the first gene in the C_H cluster (*13*). Thus the indications from all available experiments strongly favor the gene order proposed by Honjo and support his hypothesis that genomic deletion mediates the class switch in antibody-secreting cells. However, it would be difficult to account for simultaneous double production of two classes sharing a single V gene if one of the C_H genes had to be deleted before the other could be activated. A different explanation must be sought for this aspect of antibody class expression.

A first step toward understanding the molecular mechanism of δ and μ chain expression would be to determine the structure of the $C\delta$ gene and its relationship to the $C\mu$ gene in germ-line DNA. In this article we present the results of molecular cloning of both $C\mu$ and $C\delta$ genes from BALB/c mouse DNA. We find that $C\delta$ is located only about 2 kbp on the 3′ side of the membrane terminal exon of the $C\mu$ gene. Thus $C\mu$ and $C\delta$ are much closer to one another in the chromosome than are the other C_H genes mapped to date. The distance, in fact, is shorter than the intron of approximately 8 kbp that separates the exonic cluster coding for the carboxyl end of variable portion of heavy chains (the J_H region) from the $C\mu$ gene (*13*). These results suggest a model with a single manageable transcript containing one V_H region and both $C\mu$ and $C\delta$ regions, which could be spliced in several ways to yield mRNA for μ or δ chains in membrane, secretory, and possibly other forms.

Materials and Methods

In the past, analysis of the $C\delta$ gene has been hindered by the lack of a suitable nucleic acid probe for hybridization. Recently, Finkelman *et al.* (*14*) identified two previously untyped plasmacytomas, TEPC 1017 and TEPC 1033, as producers of protein that reacts with a monoclonal antibody against IgD (*15*). They showed by several methods that this protein shares antigenic determinants with the IgD present on the membranes of splenic B lymphocytes. To obtain a $C\delta$ probe we prepared a complementary DNA (cDNA) clone of reverse-transcribed mRNA from TEPC 1017 (*16*) in plasmid vector pBR322. This probe, pδ54J, was initially used to screen shotgun collections (*13*) prepared from partially digested Eco RI fragments of BALB/c liver DNA cloned into the λ bacteriophage vector Charon 4A (*17*). Due to the unfavorable distribution of Eco RI sites in the genomic DNA, we never succeeded in cloning a fragment containing both $C\mu$ and $C\delta$ in this vector.

A New Cloning Vector

For this project, a new cloning vector, Charon 28 (*18*), was developed (Fig. 1). The chief feature of this new vector is the ability to use Bam HI cloning sites in the vector to clone partial Mbo I digests of target mouse DNA. The six-base recognition site of Bam HI includes within it the four-base recognition site of Mbo I, and both enzymes cleave DNA to leave the same cohesive terminal (Fig. 1). Use of Mbo I to cut the target DNA permits generation of a wide spectrum of overlapping fragments, making it unlikely that any region of DNA would be impossible to clone.

Isolation of Genomic Cδ Clones

To prepare a Charon 28 shotgun collection, fragments obtained by partial Mbo I digestion of BALB/c DNA were fractionated by velocity sedimentation through NaCl gradients and cloned in Charon 28 (*19*). Screening by the megaplate method (*20*) with the pδ54J probe yielded several positive clones, and two, Ch28 257.3 and Ch28 257.4, were extensively characterized. We also characterized the $C\mu$ clone Ch4A142.7 obtained from a partial Eco RI fragment shotgun collection in Charon 4A (*13*).

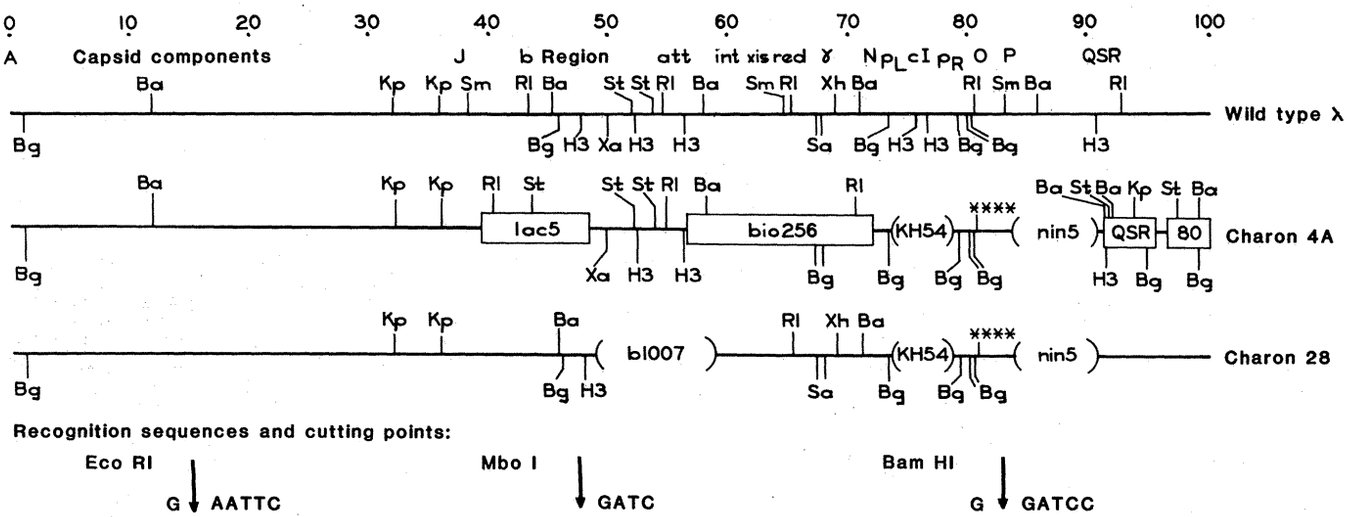

Fig. 1. The map of new cloning vector (*18*) Charon 28 is shown beneath bacteriophage λ and the original Charon 4A maps. The new vector permits cloning of Mbo I–cut target DNA into Bam HI sites in the vector. Charon 4A is suitable only for cloning Eco RI target fragments. The recognition specificities of the enzymes are shown at the bottom. Note that there is only one Eco RI site in Charon 28 DNA. A second Eco RI site expected between the Kpn I sites and the Bam site on the left arm of the vector was unexpectedly lost during construction of the vector. Abbreviations: *Ba*, Bam HI, *Bg*, Bgl II; *H3*, Hind III; *Kp*, Kpn I; *RI*, Eco RI; *Sa*, Sal I; *Sm*, Sma I; *St*, Sst I; *Xa*, Xba I; and *Xh*, Xho I.

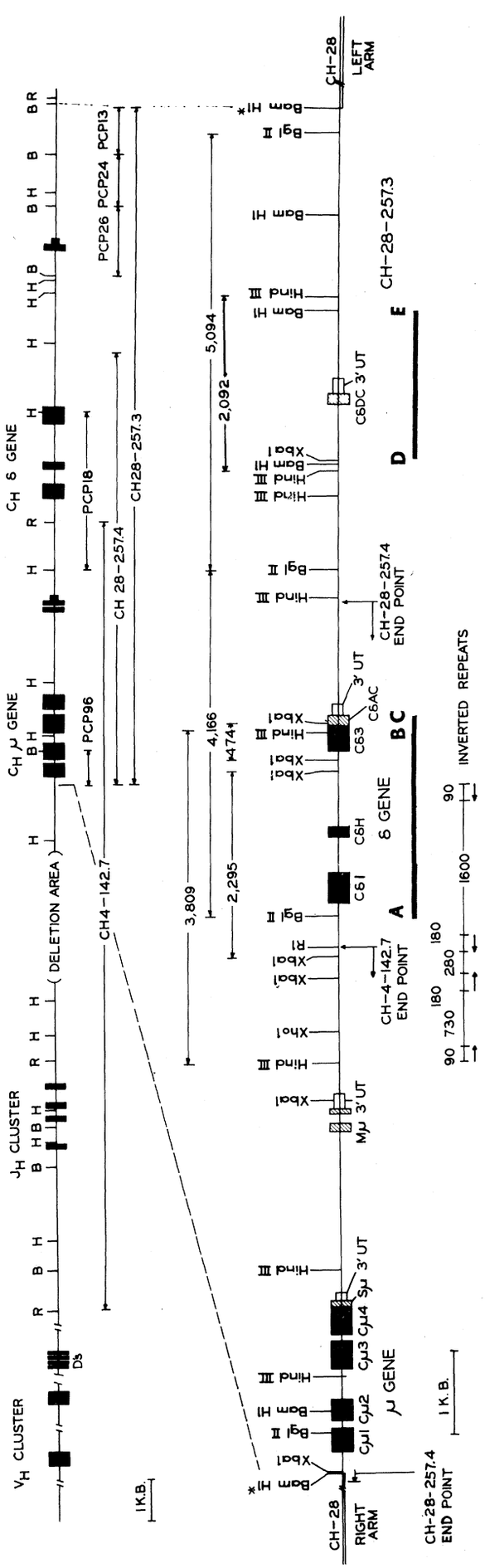

Fig. 2. Physical map of a 30-kbp portion of the Ig H chain locus from BALB/c mouse. The upper map shows the J cluster, the Cμ gene including the membrane-binding exon, and known portions of the Cδ gene drawn to scale. Restriction endonuclease sites Hind III, Bam HI, and Eco RI are designated *H*, *B*, and *R* in the upper portion. The V_H cluster and the cluster of D segments are believed to be located to the left and separated from J_H by an unknown distance. Arrows beneath the upper map show the recombinant DNA clones, derived from liver DNA of BALB/c mouse, that were used in this study. Subclones in pBR322 are designated with *PCP*. "Deletion area" designates a 2900-bp DNA region including repetitive DNA that is missing in clone Ch4A142.7 due to recombination during propagation of the clone in *E. coli*. An enlarged and more detailed map of the DNA of Ch28 257.3 is shown in the lower portion of the figure. DNA fragments showing positive hybridization to Cδ gene sequences are designated with arrows above the map showing fragment size in base pairs. The bars beneath the map show the two hybridizing regions that have homology with the Cδ probes used in Fig. 3; A, B, C, D, and E on the bars refer to the restriction sites directly above. The specific positions of δ exons within these fragments are taken from the accompanying article (*26*). The positions of inverted repeats are indicated beneath the map. End points of Ch4A142.7 and Ch28 257.4 on the Ch28 257.3 map are indicated with arrows. Bam HI sites designated with * may be due to cloning junctions in the vector and may be absent in genomic DNA.

Mapping the Cμ-Cδ Genomic Region

The structures of these clones are detailed in the maps in Fig. 2. The top line shows the entire 30-kbp segment of mouse DNA included in the three clones that contain the genes for Cμ and Cδ and the J_H cluster. The DNA inserts in the Charon phage clones are both in the u orientation (*17*), that is, the left vector arm is adjacent to the 3′ end of the cloned immunoglobulin gene region. Since the Mbo I shotgun collection was not replicated in bulk before screening for single plaques, both of the Charon 28 clones (and the Charon 4A clone) result from independent cloning events. In the regions where they overlap, all three clones are identical when compared either by restriction mapping or by heteroduplex analysis in the electron microscope (data not shown); Ch28 257.3 includes a longer stretch of genomic DNA than Ch28 257.4 does. The left cloning points of these clones are very close, and we could not determine which clone overlaps which in that area, although they differ in that a Bam HI site is present in Ch28 257.3 and absent in Ch28 257.4 (see below). Analysis of all clones was helpful for defining the detailed restriction map shown in the bottom portion of Fig. 2. This map was obtained from measurements of 53 restriction fragments on agarose and acrylamide gels obtained by single and multiple digestion of Ch28 257.3 and Ch28 257.4. Some of these fragments were subcloned into pBR322 for further mapping, as indicated in Fig. 2. This was useful in some cases to resolve the order of closely spaced restriction sites. A computer was used to calculate the best-fit map by a least-squares method (*21*). The computed numerical locations of restriction sites are listed in (*22*).

The two Bam HI sites designated by an asterisk in Fig. 2 define the ends of the cloned segment in Ch28 257.3. The one at the left is definitely not in genomic DNA or in clones Ch28 257.4 or Ch4A142.7 and is probably the result of the fusion of Mbo I site into the Bam HI site of the vector. We do not know whether the Bam HI site at the right end of the clone is also a result of cloning or whether it is indeed in the genome.

One way that we established the order of Cμ and Cδ in genomic DNA was by analyzing the clones Ch4A142.7, and Ch28 257.4 by plaque hybridization with radioactive probes for Cμ (*13, 23*) and Cδ, with the vector as a negative control. We found positive hybridization signals for the Cμ probe with both genomic clones, but only Ch28 257.4 reacted with

Cδ. In light of the map (Fig. 2), this result shows that Cδ is on the 3′ side of Cμ in genomic DNA [see (*24*)].

Which Regions of Cδ Code for TEPC 1017 Messenger RNA?

The Southern hybridization technique (*25*) was used to identify more finely which fragments of Ch28 257.3 and Ch28 257.4 hybridized to Cμ and Cδ probes. In one series of experiments of this type, we used a probe made by direct ^{32}P-labeling of TEPC 1017 mRNA with polynucleotide kinase (Fig. 3). This RNA was purified by adsorption to oligo-(dT)-cellulose columns and sucrose gradient centrifugation; it corresponded in size with the major δ mRNA of the tumor. Other experiments done with the cloned cDNA in pδ54J gave identical results. However, the mRNA probe is most informative, since it is full length, unlike the cloned cDNA probe, and we would thus expect a signal for all exons in genomic DNA that are expressed strongly in the tumor. Both probes show a strong hybridization to a number of bands in the gels (Fig. 3). Interpretation of this pattern with respect to the restriction map can be summarized as follows. Two regions of hybridization were found about 3 kbp apart; they are underlined in Fig. 2. One of these regions is bounded on the left by the Bgl II site denoted A in Fig. 2 and on the right by Xba I site C. The other region is between Xba I site D and Bam HI site E. No hybridizing region was found in the interval of DNA between sites C and D in Fig. 2.

Detailed restriction mapping and DNA sequencing of the hybridized subfragments of Ch28 257.3 (*26*) showed that the two major C region domains of the TEPC 1017 δ mRNA plus the hingelike segment between them are coded in the leftmost of the hybridizing regions of Fig. 2, whereas the hybridizing region on the right includes an exon for the 26 amino acids at the carboxyl terminus of the TEPC 1017 δ chain. The position of the Cμ gene and its restriction map agreed with published data (*27*).

Distance from Cμ to Cδ

The placement of the Cδ1 and Cδ3 domains (Fig. 2) is based on sequence data derived from Ch28 257.4 and pδ54J DNA (*26*). The Hind III site B lies within the Cδ3 domain. DNA sequence analysis from this site in both cDNA and genomic DNA shows that the orientation of the Cδ gene is the same as that of the Cμ.

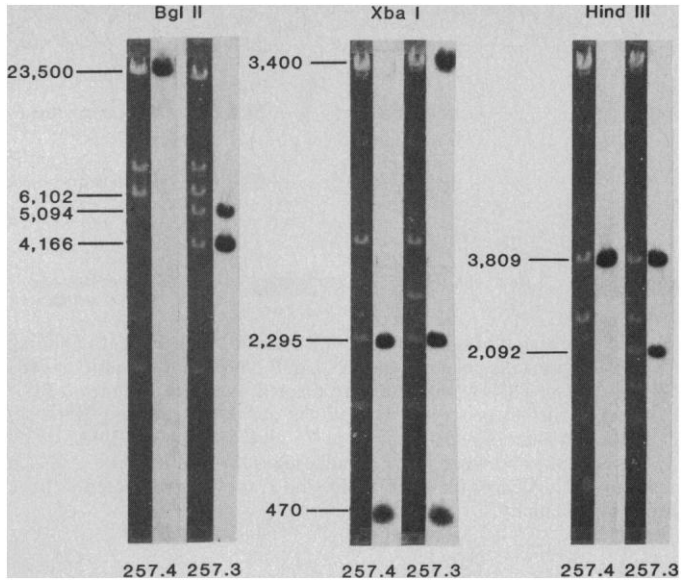

Fig. 3. DNA from Ch28 257.3 and Ch28 257.4 were digested with restriction enzymes Bgl II, Xba I, and Hind III, then subjected to Southern hybridization (*25*) and hybridized to ^{32}P-labeled δ mRNA from TEPC 1017. For each enzyme digestion, the photograph of agarose gel is on the left; the photograph of the x-ray autoradiograph, reduced to the same scale as the gel photograph, is on the right.

Placement of Cδ1 145 base pairs (bp) to the right of Bgl II site A is confirmed both by hybridization and sequence results. Thus we can estimate the distance from the carboxyl terminus of the membrane exon of Cμ to Cδ1 in the two genomic clones as 2466 bp (*28*). To extrapolate this result to the genome, additional confirmation is needed, since it is possible for a segment of DNA to be deleted or otherwise rearranged during propagation in *Escherichia coli*. [The region denoted "deletion area" between J_H and Cμ (Fig. 2) is an example of a highly reiterated segment that has shown this tendency (*13*).] We therefore did a series of Southern hybridization experiments with the pδ54J probe to determine the sizes of the Bgl II and Hind III DNA fragments in uncloned mouse liver DNA. We found both digests yielded fragments whose sizes agreed perfectly (± 10 percent) with the sizes found in the clones (data not shown). It would be quite unlikely for three independent clones to have the same structure if serious cloning artifacts were present, but small deletions of regions of a few hundred base pairs could have gone undetected by our methods.

That no hybridization of polyadenylated TEPC 1033 mRNA, TEPC 1017 δ mRNA, or pδ54J cDNA has been observed to the left of the Bgl II site A is significant. Since only the 145 nucleotides separate that site from the first Cδ domain, we conclude that no part of the Cδ gene that is transcribed and transported to the cytoplasm of the two plasmacytomas can be coded by DNA located to the left of that Bgl II site. Thus, the exon is probably the first coding segment of Cδ, namely, Cδ1.

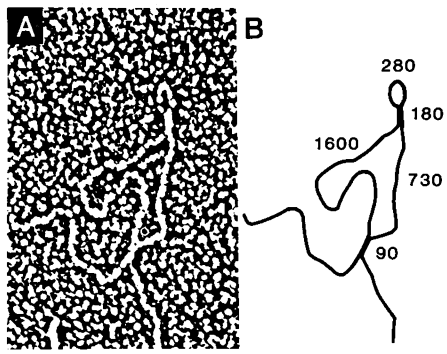

Fig. 4. Inverted repeats around the δ gene. (A) representative electron micrograph of the single strands of Ch28 257.4 DNA. (B) Interpretative drawing with the average measurements of each DNA segment. The orientation of the asymmetric double palindromic structure is indicated on Fig. 2.

Inverted Repeats Near and Within Cδ

The structure of the DNA in Ch28 257.4 and Ch28 257.3 has been examined by electron microscopy. A characteristic double-foldback structure, frequently observed in single-stranded DNA, implies the presence of two pairs of DNA segments with inverted complementary sequences. An electron micrograph showing this structure and indicating the sizes of the DNA segments is shown in Fig. 4. When heteroduplexes between Ch28 257.4 and Ch4A142.7 were examined, the small loop could occasionally be observed at the end point of Ch4A142.7 DNA just to the left of Cδ1. Thus the central pair of inverted repeats is located near the immediate 5′ side of the Eco RI site defining the right end of the Ch4A142.7 clone (see Fig. 2). The orientation of the outer pair of inverted repeats was determined by the observa-

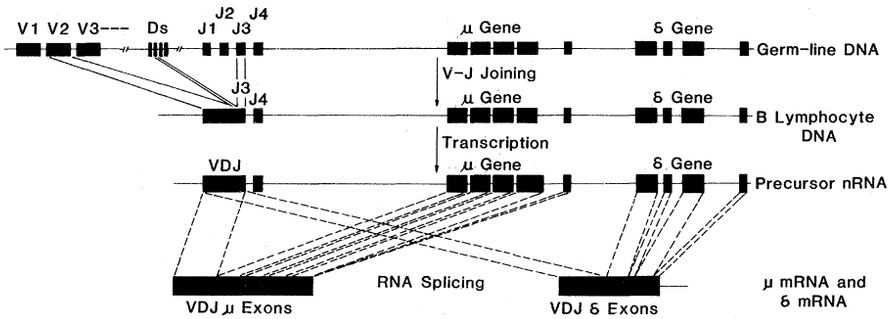

Fig. 5. Proposed model for dual expression of IgM and IgD in B lymphocytes. First, the joining of a V gene, a D segment, and a J region takes place in the DNA of B lymphocyte precursor cells to produce the V gene. The intervening DNA segments are deleted during the process. RNA precursor molecules are made and further processed by splicing out the intervening RNA sequence between J and μ and a sequence downstream of μ (to give rise to μ mRNA) or by splicing out intervening RNA sequences between J and δ including μ (to give rise to δ mRNA). Because of these two classes of mRNA carry identical V, D, and J, they are expected to have the same idiotype and antigenic specificity.

tion that the complete double-foldback structure could be seen in single-stranded DNA of plasmid DNA PCP13 (see Fig. 2), possible only if the entire structure was situated as shown. It is interesting that this assignment places one of the repeated DNA segments in the intron between the hinge and Cδ3, suggesting that the gene lies on the longer (1600-nucleotide) single-stranded DNA segment in Fig. 4. It would be interesting to speculate that these repeats may play a role in some aspect of Cδ expression.

Conclusions

We have shown that, in mouse liver DNA, genes for the μ and δ constant regions are in a cluster, with Cδ at the 3′ side of Cμ. The distance between Cμ and Cδ is extremely short (2466 bp) and therefore far less than the distance from J_H to Cμ. The Cδ gene is closely linked to a pair of inverted repeats, one of which may be in an intron between Cδ domains.

The surface IgD and IgM molecules on a given lymphocyte show identical antigenic specificity and idiotype (*3*). The DNA deletion mechanism that successfully explains the class switch appears to be unsuited to explain this dual expression. Three other possible mechanisms for producing two classes of immunoglobulins with apparently identical V regions can be considered: (i) A long-lived μ mRNA molecule could persist after transition of the cell from μ to δ expression. (ii) There could be a duplication of the V region DNA leading to assembly of two complete genes. (iii) The dual expression could be determined by processing the mRNA precursor without DNA rearrangement. The first hypothesis appears to be eliminated by the observation of cultured lymphoma cell lines (*29*) that produce both IgD and IgM of the same idiotype, even after long periods in culture. The hypothesis of V gene duplication and translocation goes back to the early history of immunology (*30*), but has not yet been supported experimentally. Our finding that the distance between μ and δ is only about 2.5 kbp, which is smaller than the J to Cμ segment known to be spliced, keeps the splicing argument as strong as any other. A schematic depiction of this proposal is shown in Fig. 5.

References and Notes

1. N. L. Warner, *Adv. Immunol.* **19**, 67 (1974).
2. U. Melchey, E. S. Vitetta, M. McWilliams, J. Philips-Quagliata, M. E. Lamm, J. W. Uhr, *J. Exp. Med.* **140**, 1427 (1974).
3. E. Abney and R. M. E. Parkhouse, *Nature (London)* **252**, 600 (1974).
4. R. L. Coffman and M. Cohn, *J. Immunol.* **118**, 1806 (1977).
5. E. Abney, I. R. Hunger, R. M. E. Parkhouse, *Nature (London)* **259**, 404 (1976); F. D. Finkelman, V. L. Woods, A. Berning, I. Scher, *J. Immunol.* **123**, 1253 (1979); A. Bargellesi, G. Corte, E. Cosulch, M. Ferrarini, *Eur. J. Immunol.* **9**, 490 (1979); J. Radl, P. Van Den Berg, C. M. Jol-Van Der Zijde, *J. Immunol.* **124**, 2513 (1980).
6. P. B. Williams, R. T. Kubo, H. M. Grey, *J. Immunol.* **121**, 2435 (1978); F. W. Alt, A. L. M. Bothwell, M. Knapp, E. Siden, E. Mather, M. Koshland, D. Baltimore, *Cell* **20**, 293 (1980); P. Singer, H. Singer, A. W. Williamson, *Nature (London)* **285**, 294 (1980).
7. J. Rogers, P. Early, C. Carter, K. Calame, M. Bond, L. Hood, R. Wall, *Cell* **20**, 303 (1980); P. Early, J. Rogers, M. Davis, K. Calame, M. Bond, R. Wall, L. Hood, *ibid.*, p. 313.
8. T. Honjo and T. Kataoka, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2140 (1978).
9. T. H. Rabbits, A. Forster, W. Dunnick, D. L. Bently, *Nature (London)* **283**, 351 (1980).
10. S. Cory and J. M. Adams, *Cell* **19**, 37 (1980); Y. Yaoita and T. Honjo, *Biomed. Res.*, in press; T. Kataoka, T. Kawakami, N. Takahashi, T. Honjo, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 919 (1980); C. Coleclough, C. Cooper, R. P. Perry, *ibid.*, p. 1422.
11. T. Honjo, personal communication.
12. S. Tonegawa, personal communication.
13. N. Newell, J. E. Richards, P. W. Tucker, F. R. Blattner, *Science* **209**, 1128 (1980).
14. F. D. Finkelman, S. W. Kessler, J. F. Mushinski, M. Potter, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 481 (1980).
15. V. T. Oi, P. P. Jones, J. W. Goding, L. A. Herzenberg, L. A. Herzenberg, *Curr. Top. Microbiol. Immunol.* **81**, 115 (1978).
16. J. F. Mushinski, F. R. Blattner, J. D. Owens, F. D. Finkelman, S. W. Kessler, L. Fitzmaurice, M. Potter, P. W. Tucker, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
17. B. G. Williams and F. R. Blattner, *J. Virol.* **29**, 555 (1979); J. R. deWet, D. L. Daniels, J. L. Schroeder, B. G. Williams, K. Denniston-Thompson, D. D. Moore, F. R. Blattner, *ibid.* **33**, 401 (1980).
18. D. Rimm, D. Horness, J. Kucera, F. R. Blattner, in preparation. Charon 28 was constructed by D. Rimm, D. Horness, and J. Kucera, undergraduate students at the University of Wisconsin.
19. Mouse liver DNA was prepared by the procedures described in (*20*). Approximately 375 μg of liver DNA was digested with 90 units of Mbo I (New England Biolabs) at 37°C in 75 m*M* NaCl, 10 m*M* tris-HCl (*p*H 7.4), 10 m*M* $MgCl_2$, and 1 m*M* dithiothreitol. At 10, 20, and 30 minutes of digestion, one-third of the volume of the reaction sample was taken, and digestion was stopped by adding EDTA to a final concentration of 20 m*M*; the enzyme was inactivated by adding diethyl pyrocarbonate to a final concentration of 0.1 percent. The three samples were combined and layered onto a 12-ml 5 to 20 percent NaCl gradient and centrifuged in a Beckman SW41 rotor at 35,000 rev/min at 25°C for 4 hours. Twenty fractions were collected and 10 μl of each fraction was taken and subjected to electrophoresis in a 0.6 percent agarose gel. Fractions that contained fragments of 8 to 20 kb were pooled, and alcohol was added to a 70 percent concentration to precipitate DNA molecules. The DNA pellet was dissolved in 10 m*M* tris-HCl, *p*H 7.9, and 1 m*M* EDTA. These Mbo I DNA fragments were subsequently ligated with Charon 28 arms to generate recombinant DNA molecules. Charon 28 arms were prepared as follows: Charon 28 DNA at a concentration of 1 mg/ml was ligated at the cohesive ends with T4 DNA ligase, then digested with Bam HI enzyme. The digested DNA was loaded onto a 12-ml, 5 to 20 percent NaCl gradient and centrifuged at 35,000 rev/min at 25°C for 4 hours. Twenty fractions were collected; fractions containing the 32-kb arms were pooled, and DNA was precipitated with alcohol. The Charon 28 arm pellet was dissolved in 10 m*M* tris-HCl, *p*H 7.9, and 1 m*M* EDTA at a concentration of 1 mg/ml. The liver DNA fragments and the Charon 28 arms were mixed and ligated at a vector-to-target ratio of 3:1 (by weight). The overall DNA concentration was 650 μg/ml. Packaging of the ligated DNA in vitro was performed to generate viable recombinant phages by the method described in (*20*).
20. F. R. Blattner, A. E. Blechl, K. Denniston-Thompson, H. E. Faber, J. E. Richards, J. L. Slightom, P. W. Tucker, O. Smithies, *Science* **202**, 1279 (1978).
21. J. L. Schroeder and F. R. Blattner, *Gene* **4**, 167 (1978).
22. The restriction enzyme sites and their map coordinates (nucleotides) are: Bam HI*, 0; Xba I, 6; Bgl II, 516; Bam HI, 828; Hind III, 1,170; Hind III, 2,149; Xba I, 4,457; Hind III, 5,085; Xho I, 5,263; Xba I, 5,812; Xba I, 6,135; Eco RI, 6.173; Bgl II, 6.618; Xba I, 8,430; Xba I, 8,552; Hind III, 8,894; Xba I, 9,022; Hind III, 10,519; Bgl II, 10,784; Hind III, 11,675; Hind III, 11,966; Bam HI, 12,066; Xba I, 12,098; Bam HI, 13,891; Hind III, 14,058; Bam HI, 14,969; Bgl II, 15,878; and Bam HI*, 16,181.
23. U. Schibler, K. B. Marcu, R. Perry, *Cell* **15**, 1495 (1978); K. B. Marcu, U. Schibler, R. P. Perry, *Science* **204**, 1087 (1979).
24. Pollock *et al.* recently reported that the δ gene of the New Zealand Black (NZB) strain of mouse may be on the 5′ end of the μ gene, since their serological data indicates that the NZB strain has the a allotype of δ whereas μ and the other constant regions of NZB are of the e allotype [R. R. Pollock, M. E. Dorf, M. F. Mescher, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4256 (1980)]. These data could be subjected to a variety of other interpretations. A cloning experiment on NZB DNA with the probes we have constructed might resolve this point at the molecular level.
25. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1978). The DNA from Ch28 257.4 or from Ch28 257.3 was digested with various combinations of restriction enzymes, including Xba I, Bam HI, Hind III, Xho I, Eco RI, and Bgl II, and then subjected to electrophoresis through 0.8 percent agarose gels that were stained with ethidium bromide and photographed with ultraviolet illumination. Southern transfers were made of the replicate gels, and each nitrocellulose paper was hybridized with ^{32}P-labeled δ probe or ^{32}P-la-

beled μ probe, or in some cases, with δmRNA that was labeled with ^{32}P by using polynucleotide kinase as described by N. Maizels, *Cell* **9**, 431 (1976).
26. See the article by P. W. Tucker, C.-P. Liu, J. F. Mushinski, F. R. Blattner, *Science* **209**, 1353 (1980).
27. N. M. Gough *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 554 (1980).
28. To calculate this, we first calculated the distance from the Xba I site in the 3′ untranslated region of the μ membrane exon to the Bgl II site just to the left of Cδ1 by subtracting the coordinates (*22*) (6618 − 4457 = 2161). We then added to this value 145 bp for the Bgl II to Cδ1 distance and 160 bp for the distance from the Cμ exon terminator to the Xba I site (*7*). The result was 2466 nucleotide pairs.
29. S. Slavin and S. Strober, *Nature (London)* **272**, 624 (1978); M. R. Knapp, E. Severison-Gronowicz, J. Schroeder, S. Strober, *J. Immunol.* **123**, 1000 (1979); M. Knapp, personal communication regarding the observation that IgM and IgD molecules on BCL-1 lymphoma cells show the same idiotype.
30. W. J. Dreyer and J. C. Bennett, *Proc. Natl. Acad. Sci. U.S.A.* **54**, 865 (1965).
31. We thank M. Fiant and J. Richards for work on heteroduplex and electromicroscopy, E. Lui for technical help on phage plaque purification, and W. Fiske and E. La Luzerne for manuscript preparation. All experiments were carried out in accordance with the NIH Guidelines on Recombinant DNA Research. Supported by grants GM 21812 (F.R.B.) and GM 06768 (C.P.L.) from the National Institute of General Medical Sciences, and by grant AI 16547 (P.W.T.) from the National Institute of Allergy and Infectious Diseases. This is paper number 2447 from the Laboratory of Genetics, University of Wisconsin.

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Mouse Immunoglobulin D: Messenger RNA and Genomic DNA Sequences

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Summary. The molecular structure of a mouse immunoglobulin D from a plasmacytoma tumor and that of the normal mouse gene coding for immunoglobulin D are presented. The DNA sequence results indicate an unusual structure for the tumor δ chain in two respects: (i) Only two constant (C) region domains, termed Cδ1 and Cδ3 by homology considerations, are found; the two domains are separated by an unusual hinge region CδH that lacks cysteine residues and thus cannot provide the covalent cross-links between heavy chains typically seen in immunoglobulins. The two domains and hinge are all coded on separate exons. (ii) At the carboxyl end of the δ chain there is a stretch of 26 amino acids that is coded from an exon located 2750 to 4600 base pairs downstream from the rest of the gene. Analogy with immunoglobulin M suggests that this distally coded segment CδDC may have a membrane-binding function; however, it is only moderately hydrophobic. A fifth potential exon (CδAC), located adjacent to the 3′ (carboxyl) end of Cδ3, could code for a stretch of 49 amino acids. The tumor's expression of the δ gene may be aberrant, but the simplest interpretation would be that this tumor expresses one of the several biologically significant forms of the δ chain.

Immunoglobulin molecules on the membranes of lymphocytes serve as receptors and in this form play a pivotal role in regulating the immune response of an animal to antigenic challenge (*1*). One example is the antigen-dependent triggering of B cells (*2*). To be eligible to differentiate into a proliferating antibody-secreting cell, a particular B cell must produce and bear on its surface an immunoglobulin that can react with the antigen. Thus the animal's protein-synthesizing resources are not devoted to large-scale synthesis of useless antibodies for which an antigen is not present.

Superimposed on this regulatory process are additional controls that prevent triggering of B cells bearing self-reactive antibodies (*3*) and, in the case of cellular antigens, histocompatibility restrictions that regulate T cell–B cell interactions (*4*, *5*).

Immunoglobulin D (IgD) whose role is at present poorly understood, is a major immunoglobulin receptor on B cell membranes (*6*). The IgD class is distinguished by its δ type heavy chains. Membrane expression of IgD may be associated with such functions as memory propagation (*7*) and maturation (*8*), prevention of tolerance (*9*), idiotype suppression (*10*), and early B cell development (*11*). Although IgM appears first in ontogeny (*12*, *13*), IgD is present along with IgM on a majority of splenic B lymphocytes (*12*, *14*). On each cell these two classes of receptor share the same antigenic specificity and idiotype (*15*), which probably means that they have the same variable region.

Most of the available knowledge about immunoglobulin structure has come from analysis of plasmacytoma proteins, which are assumed to reflect the secreted rather than the membrane form of antibodies. Both types of immunoglobulin molecules are generally composed of paired heavy (H) and light (L) chains, each of which is made up of a series of domains of about 110 amino acid residues. Both heavy and light chains contain a variable (V) region domain at the amino terminal end; the heavy chain and light chain V regions interact to form the antigen-combining site. Light chains have a single constant (C_L) region domain located at the carboxyl terminus which is linked to the first constant region domain of the heavy chain (C_H1). In all normal secreted immunoglobulins so far studied, heavy chains have either three or four C_H domains depending on class. The heavy chains interact with each other covalently by disulfide bonds and noncovalently by other interactions to form an H_2L_2 tetramer (which in immunology is traditionally termed a monomer) of characteristic three-dimensional structure (*16*). In IgM and IgA, the secreted form of these monomers are further polymerized. In the IgG class, a peptide segment termed the hinge is present between the first and second C_H domains. This segment of amino acids is generally rich in proline, is involved in disulfide bonding between heavy chains,

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