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Immunology

Simultaneous expression of immunoglobulin μ and δ heavy chains by a cloned B-cell lymphoma: A single copy of the V_H gene is shared by two adjacent C_H genes

(lymphocyte tumor/DNA rearrangement/IgM/IgD)

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ABSTRACT **The cloned murine B-cell lymphoma line (BCL_1) that expresses surface IgM and IgD is considered to be a model for the immunoglobulin gene expression of the mature virgin B cell. Of particular interest is the mechanism by which a single V_H gene is shared by two C_H genes. We examined the organization of the immunoglobulin heavy chain genes in BCL_1 DNA. A single arrangement of C_H genes was found with the expressed V_HDJ_H gene complex just 5′ to the C_μ gene. The complete DNA sequence of the V_H gene was determined. No rearrangement occurred in the intervening DNA between the J_H and C_μ genes or between the C_μ and C_δ genes. We conclude that dual expression of μ and δ heavy chains using a single V_H gene is accomplished by alternate processing of a primary transcript that encompasses the V_HDJ_H complex and both C_H genes.**

The locus coding for immunoglobulin heavy chains in the mouse contains a series of V genes (1, 2), D segments (3), four J_H segments (4, 5), and eight tandemly arranged C_H genes corresponding to the immunoglobulin class (6). A great deal has been learned about the expression of antibody genes through the study of DNA from mouse plasma cell tumors that represent a clonal expansion of a single antibody-secreting cell (7). At least two types of gene rearrangements that can be studied in plasma cell tumors (5, 8, 9) can occur by the time the B cell has reached the terminal differentiation state. The first involves translocation of a given V_H gene (selected from a pool of several hundred genes) to the immediate 5′ side of the J_H genes with the inclusion of a short D segment between them. The second immunoglobulin gene rearrangement is observed in plasmacytomas secreting immunoglobulins other than IgM. The latter rearrangement is associated with the deletion of all the C_H genes 5′ to the expressed C_H gene (2, 6, 10, 11). Thus, the first C_H gene to the right of the V_HDJ_H complex is the one expressed in a plasmacytoma. This evidence strongly supports the concept of a deletional "class switch" mechanism originally propounded by Honjo and Kataoka (6).

The expression of immunoglobulins by virgin and memory B cells (plasma cell precursors) is complex, since the majority of B cells bear IgM and IgD on their surface (12, 13) but do not secrete immunoglobulin. Dual expression seems difficult to explain by the deletion model of Honjo and Kataoka.

In this communication, we extend the molecular analysis of immunoglobulin gene expression to a cloned B-cell lymphoma line (BCL_1) that shows many of the characteristics of the mature virgin B cell and expresses both surface IgM and IgD (14–17). Our goal was to determine whether any DNA rearrangement of the immunoglobulin heavy chain genes other than V–D–J joining had occurred in this tumor. In particular, we investigated the flanking sequences of the C_μ and C_δ genes to discover whether a V_H gene was located between C_μ and C_δ as well as 5′ to the C_μ gene. The studies of BCL_1 cells presented here show that the germ-line arrangement of C_μ and C_δ genes is preserved in this dual-expressing cell. A V_H gene is found 5′ to the C_μ gene, but no other V_H gene is located between the C_μ and C_δ genes.

MATERIALS AND METHODS

Cell Lines, Culture Conditions, and Sera. The cloned BCL_1 5b13 line used was derived by limiting dilution of the uncloned BCL_1 5b line established from the parent BCL_1 tumor of BALB/c mouse origin (17). Anti-idiotype serum was derived by immunization of Lewis rats with BCL_1 antibody of IgM class purified from the hybrid cell line BCL.F2/8 derived by fusion of BCL_1 tumor cells to the myeloma NS-1 (16). Constant region activities were removed by extensive absorption with Sepharose 4B-bound MOPC 104E myeloma protein (μ, λ) and normal mouse serum. Monoclonal anti-δ (10-4.22) (18) and specific anti-λ reagents have been described (14).

Cell Surface Protein Labeling. ^{125}I labeling of the surface proteins of (BCL_1) cells was carried out by using the lactoperoxidase procedure (19). Lysates were incubated with excess antisera for 30 min at 37°C and 1–18 hr at 4°C. Immune complexes were adsorbed to heat-killed formalin-fixed *Staphylococcus aureus* (The Enzyme Center, Boston, MA) for 15 min at room temperature, collected by centrifugation, and eluted as described (19). In some experiments, μ chains were removed from lysates before immunoprecipitation by absorption with anti-μ-coated *S. aureus*. Immunoprecipitated samples were subjected to electrophoresis through 10% polyacrylamide/$NaDodSO_4$ gels (20), and ^{125}I-labeled proteins were visualized by autoradiography.

DNA Clones. Plasmids pδ54J (δ cDNA) (21) and pNN12 (germ-line J_{H1} and J_{H2}) (4) have been described. Plasmid pMK-1 (μ cDNA) was constructed from BCL.F2/8 RNA by the technique of Maniatis *et al.* (22). Plasmid pV_H3 (BCL_1 variable region) was a 1-kilobase *Bam*HI fragment from phage CH 28-289.1 subcloned in pBR322. Plasmids to be used as hybridization

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probes were labeled with ^{32}P by nick-translation (23). Shotgun collections of genomic fragments derived by partial *Mbo* I digestion of DNA from BCL_1 5b13 cells were made in Charon 28 (24, 25). Recombinant phage Ch28-288.1 (C_μ,C_δ), Ch28-289.1 (V_H,C_μ), and Ch28-291.2 (C_μ,C_δ) containing μ or δ constant region sequences were identified by plaque hybridization (26).

RESULTS

IgM and IgD on BCL_1 Cells Share Idiotypic Determinants. Like the uncloned parent BCL_1 tumor line (14, 17), all of the cells in the cloned line BCL_1 5b13 used in this study expressed IgM and IgD on the surface as judged by immunofluorescent staining with these reagents using the fluorescence-activated cell sorter (unpublished observations). Fig. 1 shows that lysates from normal spleen cells and the cloned BCL_1 line have the characteristic μ and δ heavy chain bands after precipitation with anti-λ antisera. The μ/δ ratio in normal B cells varies over a considerable range and BCL_1 has a low δ/high μ phenotype (compare Fig. 1*A*, lane 3, and Fig. 1*B*, lane 2). When the anti-idiotype reagent was used to precipitate proteins from the BCL_1 lysate directly, large amounts of μ chains were observed in the gels, but proteins migrating in the position of δ chains were too faint to be seen (data not shown). We suspected that the idiotype-specific antibodies were saturated by the highly abundant μ chains in the BCL_1 lysates so that little remained to precipitate δ chains. Therefore, cell lysates were first absorbed with anti-μ-coated *S. aureus* to reduce the level of μ chains. As seen in lane 3 of Fig. 1*B*, the anti-idiotype reagent precipitates a protein comigrating with that precipitated by the monoclonal anti-δ reagent (Fig. 1*B*, lane 1). Thus, μ and δ chains from BCL_1 cells share idiotypic determinants and, therefore, have identical or extremely similar variable regions.

Structure of the BCL_1 V_H Gene. To examine the variable region gene of BCL_1 in greater detail, this region was cloned and the nucleotide sequence (27) was determined (Fig. 2). The protein structure predicted from the DNA sequence is clearly that of a variable region of V_H subgroup II (28). The BCL_1 V_H sequence is similar (75% match) to that of the MOPC 104E μ chain V region, although BCL_1 uses the J_{H2} gene whereas MOPC 104E uses J_{H3}. A D segment of two amino acids is found in the BCL_1 variable region that is different in sequence but not in length from that of MOPC 104E (29). The sequence was confirmed by comparison with the 20 NH_2-terminal amino acid residues of BCL_1 V_H at the protein level (D. Capra, University of Texas, Southwestern Medical School, Dallas, personal communication).

Genomic Arrangement of the V_H Gene in BCL_1 DNA. The nucleotide sequence shown in Fig. 2 predicts the existence of *Pst* I and *Xba* I sites in the BCL_1 V_H gene. Thus, *Pst* I and *Xba* I were used to digest BCL_1 and liver DNA for Southern blot hybridization (30) with a $J_{H1}J_{H2}$ probe. The analysis is shown in Fig. 3.

As shown in the germ-line map in Fig. 3, the $J_{H1}J_{H2}$ probe contains a single *Pst* I site located between J_{H1} and J_{H2}, and thus two *Pst* I fragments are labeled in the hybridization to liver DNA with this probe. The larger [1,200-base pair (bp)] fragment contains J_{H1} whereas the 590-bp fragment (whose sequence from germ-line DNA is completely known) contains J_{H2} and J_{H3}. A single 720-bp *Pst* I fragment observed in BCL_1 DNA is exactly the size that would be predicted by splicing the variable region of BCL_1 to J_{H2} and, as expected, the 1,200-bp germ-line fragment containing J_{H1} is missing in BCL_1 DNA. The *Xba* I digest of germ-line DNA shows a single DNA fragment since there is no *Xba* I site in the segment covered by the probe. In BCL_1 DNA, this fragment is replaced by a shorter one, as would be expected due to the introduction of the *Xba* I site in the V_H segment. Although the germ-line *Xba* I sites are not in a region for which the sequence is known, the size of the *Xba* I fragment

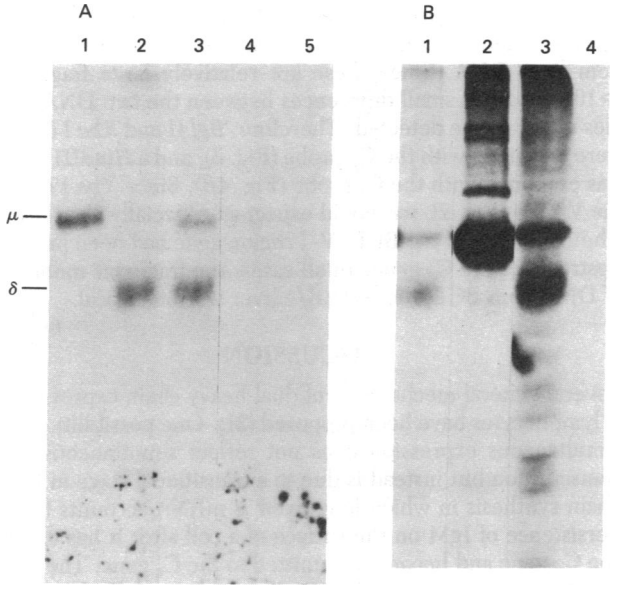

FIG. 1. Immunoprecipitation of ^{125}I-labeled surface immunoglobulins from normal spleen (*A*) and BCL_1 (*B*) cell lysates. (*A*) Lanes: 1, anti-μ reagent; 2, anti-δ reagent; 3, anti-λ reagent; 4, anti-BCL_1 idiotype; 5, normal rat serum. (*B*) Lanes: 1, anti-δ reagent; 2, anti-λ reagent; 3, anti-BCL_1 idiotype; 4, normal rat serum. Lanes 3 and 4 were precleared with anti-μ reagent. Gels are from single representative experiments. It should be noted that the monoclonal anti-δ reagent precipitates high molecular weight protein from BCL_1 that is not found with normal spleen cells.

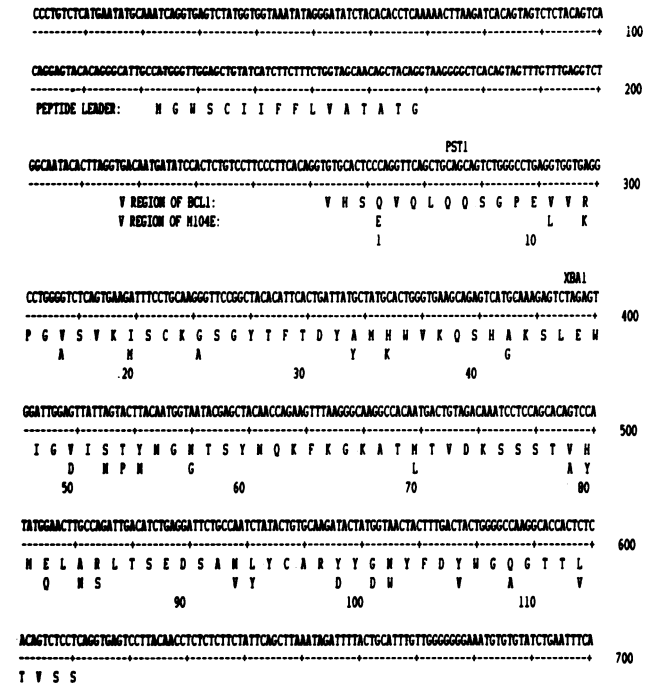

FIG. 2. Nucleotide sequence of DNA coding for the leader peptide and V_H segment of BCL_1, including the 5′ flanking sequence. The predicted amino acid sequence is shown beneath the nucleotide sequence. The numbering system and single letter amino acid abbreviations are according to Kabat *et al.* (28). Amino acids listed below the predicted sequence are found in the V_H protein of MOPC 104E. *Xba* I and *Pst* I sites are shown above the sequence.

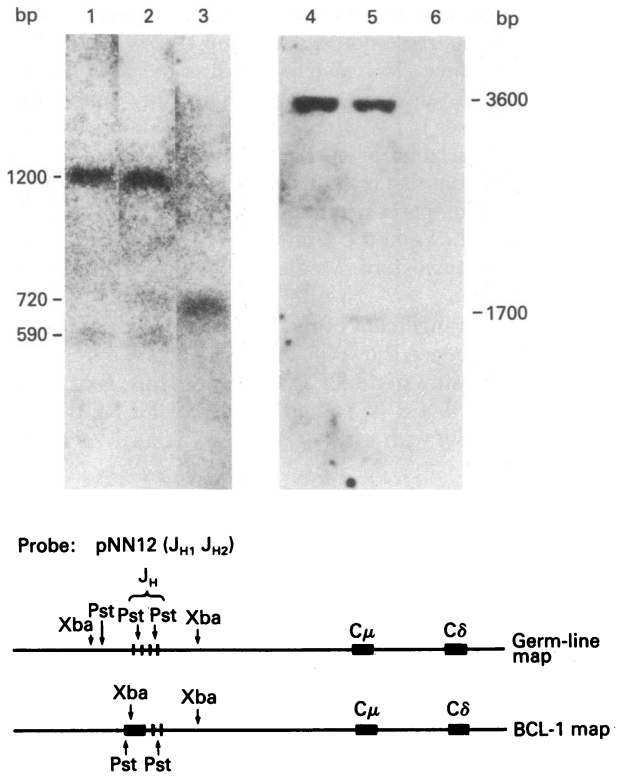

FIG. 3. Comparison of restriction fragments containing $J_{H1}J_{H2}$ genes in recombinant phage and BCL_1 genomic DNA. DNA (7.5 μg) from normal BALB/c liver (lanes 1 and 4), a mixture of 7.5 μg of normal BALB/c liver DNA and 100 pg of phage Ch28-289.1 DNA (lanes 2 and 5), and 7.5 μg of BCL_1 genomic DNA (lanes 3 and 6) were digested with *Pst* I (lanes 1–3) or *Xba* I (lanes 4–6) and probed for the presence of the $J_{H1}J_{H2}$ genes. Maps of germ-line and BCL_1 DNAs with only those sites contributing to labeled fragments are shown below. bp, Base pair(s).

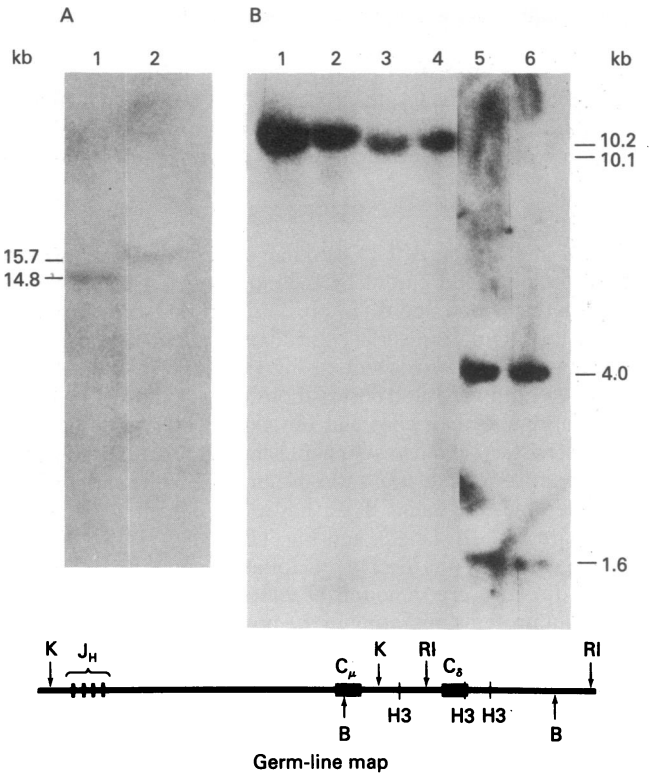

FIG. 4. Comparison of restriction fragments containing C_μ genes in liver and BCL_1 genomic DNAs. (*A*) BALB/c liver (lane 1) and BCL_1 genomic (lane 2) DNAs were digested with *Kpn* I and probed for the presence of the C_μ gene. (*B*) Liver (lanes 1, 3, and 5) and BCL_1 (lanes 2, 4, and 6) genomic DNAs were digested with *Bam*HI (lanes 1 and 2), *Eco*RI (lanes 3 and 4), and *Hin*dIII (lanes 5 and 6) and probed for the C_δ gene with plasmid pδ54. The germ-line map shows only sites leading to labeled fragments. kb, Kilobase(s).

in BCL_1 DNA is the same as that found in clone 289.1.

These data establish that the V_H found contiguous to the J_{H2} gene in Ch28-289.1 occupies the same position in the BCL_1 genome, and the absence of any other hybridization bands in the germ-line position or elsewhere indicates that there is only one copy of the V_HJ_{H2} complex in the BCL_1 genome.

To relate the V_HJ_H complex to the C_μ gene in the BCL_1 genome, BCL_1 and liver DNAs were digested with *Kpn* I, which is known to produce a single fragment from germ-line DNA that carries both C_μ and the entire J_H gene locus. The fragments were probed for C_μ sequences and, as shown in Fig. 4*A*, each DNA contained a single fragment, but mobilities were different from the two DNA sources. Identical-sized fragments were also shown to hybridize with the $J_{H1}J_{H2}$ probe (data not shown). These studies show that there is a single copy of the C_μ gene in BCL_1 DNA and that this copy is associated with the V_HJ_H gene complex for which we have determined the sequence.

The C_μ–C_δ Region in BCL_1 DNA Does Not Differ from the Germ-Line Arrangement. Clones 288.1 (C_μ, C_δ) and 291.2 (C_μ,C_δ) from the BCL_1 DNA library were mapped with restriction endonucleases and no differences from liver DNA clones were found. To directly examine the organization of the C_μ and C_δ genes in uncloned BCL_1 genomic DNA, restriction digests of BCL_1 and liver DNA were hybridized to the C_μ and C_δ probes. From the germ-line restriction map (4, 24), enzymes were chosen that would be sensitive to rearrangements of the BCL_1 DNA within or between the C_μ and C_δ genes. *Bam*HI, which cuts within the C_μ gene and on the 3′ side of the C_δ gene, generates a single fragment containing the entire region. As seen in Figs. 4*B* and 5, the *Bam*HI fragments containing the C_μ and C_δ genes in the BCL_1 DNA migrate identically to those from liver DNA. Since these are relatively large fragments (>10 kilobases), small differences between the two DNA samples might not be detected. Therefore, *Bgl* II and *Xba* I digests were examined with the C_μ probe (Fig. 5), and a *Hin*dIII digest was examined with the C_δ probe (Fig. 4*B*). Since *Xba* I cuts in the V gene (Fig. 2), we would expect an especially clear test of whether a copy of the BCL_1 V–J region gene had been inserted upstream of the C_δ gene. In all cases, the fragment mobilities of DNA from BCL_1 and BALB/c liver were identical.

DISCUSSION

Several general mechanisms of dual heavy chain expression in B lymphocytes have been proposed (31). One possibility is that simultaneous expression does not reflect simultaneous gene transcription but instead is due to a transitional stage in heavy chain synthesis in which long-lived μ mRNA accounts for the persistence of IgM on the surface of a cell after it has deleted the C_μ gene and become committed to the C_δ gene. The maintenance of a surface IgM/IgD phenotype on all cells of the cloned cell line BCL_1 5b13 for many generations argues against this possibility. It is also possible that μ and δ expression originates from genes located on different chromosomes. This mechanism is ruled out in the case of the BCL_1 because only one copy of the J_H, C_μ, and C_δ genes is present in the BCL_1 genome. The latter finding is consistent with the loss of one copy of chromosome 12, which was previously noted in the karyotype analysis of the *in vivo* BCL_1 parent line (32). Chro-

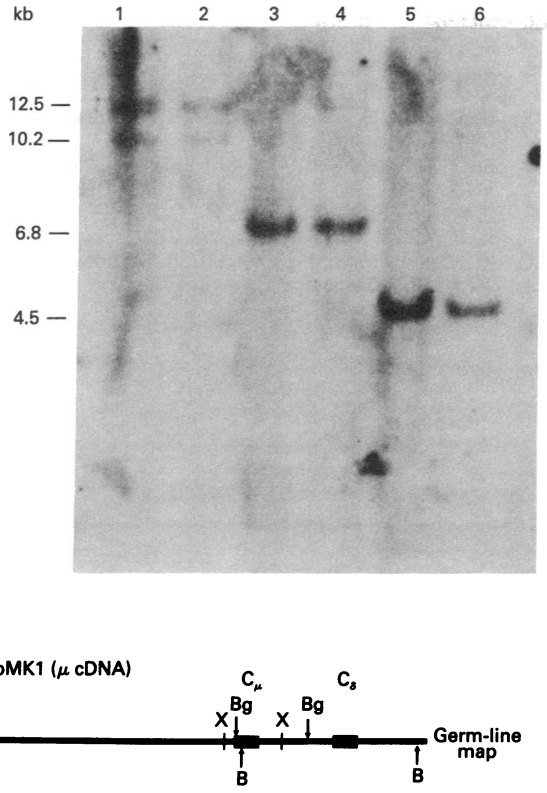

FIG. 5. Comparison of genomic contexts of the C_μ gene in BCL_1 and BALB/c liver DNAs. Liver (lanes 1, 3, and 5) and BCL_1 (lanes 2, 4, and 6) DNAs were digested with *Bam*HI (lanes 1 and 2), *Bgl* II (lanes 3 and 4), and *Xba* I (lanes 5 and 6) and probed for the C_μ gene with plasmid pMKI. Map shows relevant sites only. kb, Kilobase(s).

mosome 12 has been shown to carry the immunoglobulin heavy chain genes (33). A third potential genetic mechanism of simultaneous μ and δ synthesis might involve translocation of a copy of the V_H gene to a position between the C_μ and C_δ genes. The finding that the C_μ and C_δ genes in the BCL_1 genome are present in the germ-line context rules out this hypothesis and indicates that the C_μ and C_δ genes share the V_H gene that is 5′ to C_μ. This supports the conclusion that surface IgM and IgD share the same variable region as judged by immunoprecipitation analysis.

The only remaining plausible explanation is that μ and δ chains are expressed with a common V gene by an alternate RNA splicing mechanism. We propose that δ chains are translated from mRNA derived by processing of a primary transcript that includes sequences from both C_μ and C_δ genes. The μ mRNA may be processed either from an identical transcript or from one that terminates in the intervening sequence separating the C_μ and C_δ genes. A similar mechanism has been invoked to explain the production of two functionally distinct μ chains having different COOH-terminal amino acid sequences (34, 35). It should be pointed out that regulation of the processing of the $\mu\delta$ precursor must be quite complex to avoid aberrant mRNAs. For example, it is not clear why immunoglobulins are not produced bearing μ domains spliced to δ domains. Highly controlled regulation of splicing patterns is found in adenovirus mRNA processing. We assume that similar mechanisms are in force in both cases to avoid inappropriate fusion of exons.

It is not clear whether these mechanisms are used by B lymphocytes to express only the IgM/IgD combination or whether these are general mechanisms by which nonsecreting lymphocytes can express other combinations of surface immunoglobulin classes simultaneously, as reported by several investigators (12, 13, 36). It is reasonable to assume that other C_H genes might be translocated to a position downstream of C_μ, possibly replacing C_δ with another isotype. In this case, dual expression of other isotypes would be explained by the mechanism described here for $\mu\delta$ expression.

Thus, the immunoglobulin gene context during B-cell development may involve at least three gene rearrangements: (*i*) V_HDJ_H translocation with commitment to a single idiotype and IgM/IgD surface expression; (*ii*) a translocation at the level of the mature B cell bringing other C_H genes 3′ to the C_μ gene and deleting C_δ for dual or multiple surface immunoglobulin expression; and (*iii*) a translocation at the level of the mature immunoglobulin-secreting plasma cell that brings the C_H gene whose product is to be secreted immediately proximal to the V_HDJ_H gene complex, deleting all the intervening genes and restricting immunoglobulin secretion to a single class.

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