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Expression of IgD may use both DNA rearrangement and RNA splicing mechanisms

(IgD myeloma tumor/δ gene organization/δ gene expression)

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ABSTRACT **From a library of mouse sperm DNA, we have isolated two overlapping clones which contain the C_δ gene. One of these clones also contains the C_μ gene. The C_δ gene is separated from the C_μ membrane exons by approximately 2 kilobases (kb) of DNA. The C_δ gene was identified by (*a*) hybridization to poly(A)$^+$RNA prepared from the IgD-producing rat plasma cell tumor IR731, and (*b*) homology of a translated nucleotide sequence to the amino acid sequence of the human δ chain. The C_δ gene spans 8 kb of DNA in the germ line. Plasmid subclones of the C_δ gene were used as probes in Southern and RNA blot experiments. RNA blot analysis of cytoplasmic poly(A)$^+$RNA from IR731 and a $\mu^+\delta^+$ B-cell hybridoma revealed 1.6- and 2.7-kb δ mRNA species with different 3′ ends, which presumably encode the secreted and membrane-bound forms, respectively, of the δ chain. Southern blot analysis of DNA from two $\mu^+\delta^+$ lymphomas revealed that the C_δ gene is in the germ-line configuration in each case. Restriction map analysis of C_μ and C_δ genomic clones isolated from a library of normal $\mu^+\delta^+$ B-cell DNA also gave no evidence for DNA rearrangement in the region between the C_μ and C_δ genes. Taken together, these data suggest that IgD expression in $\mu^+\delta^+$ B cells does not involve a V_H-to-C_δ DNA switch rearrangement. We propose that simultaneous expression of C_μ and C_δ with a single V_H gene is mediated by two alternative routes of RNA processing of a primary nuclear transcript which contains the V_H, C_μ, and C_δ genes. In contrast, analogous experiments with myeloma IR731 DNA revealed that the C_μ gene has been deleted from the myeloma DNA and that the C_δ gene has undergone DNA rearrangement, presumably including a switch recombination of the V_H gene from the C_μ to the C_δ gene. These results indicate that two alternative mechanisms may be used in the expression of IgD molecules—RNA splicing in B cells and DNA rearrangement in plasma cells.**

An immunoglobulin heavy chain is composed of a variable (V_H) region and one of five classes of constant (C_H) region: C_μ (IgM), C_δ (IgD), C_γ (IgG), C_α (IgA), and C_ε (IgE). In mice, the five C_H classes are encoded by eight distinct genes: C_μ, C_δ, $C_{\gamma 1}$, $C_{\gamma 2a}$, $C_{\gamma 2b}$, $C_{\gamma 3}$, C_α, and C_ε. Early in its development, a lymphocyte (B cell) bears only IgM on its surface; later, IgD molecules are often expressed together with IgM molecules (for review, see ref. 1). Upon interaction with antigen, a B cell proliferates and differentiates, ultimately becoming a plasma cell. The class of immunoglobulin produced by its progeny may change, from IgM (and IgD) to IgG, IgA, or IgE.

The molecular mechanisms by which these genes are expressed during B-cell development have been partially characterized. The V_H gene is joined to the C_μ gene by assembly of three gene segments: *V*, *D*, and *J* (2, 3). Presumably, upon recognition of antigen, the V_H gene, along with some C_μ 5′-flanking sequence, is joined to another C region in a phenomenon called the heavy-chain switch (4–6). Honjo and coworkers (7, 8) have suggested that the intervening DNA, containing the C_μ gene and perhaps other C_H genes, is deleted.

Both IgM and IgD molecules are present on the surface of the B cell. Experiments utilizing allotypic markers have shown that their expression conforms to the rule of allelic exclusion: both heavy chains on the surface of an individual cell are encoded by the same chromosome (9). Moreover, considerable evidence suggests that these two cell-surface molecules bear identical V_H regions (10–13). These data are difficult to reconcile with the C_H gene deletion model because they imply that a V_H gene can be expressed with the C_δ gene without concomitant deletion of the C_μ gene. A number of mechanisms have been suggested to account for these observations, including the "copy-insertion" mechanism (14) in which a copy of the V_H gene is joined to the C_δ gene while the original remains joined to the C_μ gene, and differential RNA processing of a single transcript containing V_H, C_μ, and C_δ genes (15).

This paper reports experiments carried out to test these hypotheses. The results support RNA splicing as the mechanism by which the C_μ and C_δ genes are expressed simultaneously in B cells and also suggest that V_H-to-C_δ DNA switch recombination may occur in plasma cells that express only IgD.

MATERIALS AND METHODS

Rat IgD Myeloma. The rat myeloma IR731 (16), a plasmacytoma, was passaged subcutaneously in Lou/M/Wsl N rats (NIH). Total cell poly(A)$^+$RNA was prepared from IR731 by a method similar to that of Chirgwin *et al.* (17), followed by oligo(dT)-cellulose chromatography. This RNA was hydrolyzed with base to an estimated average size of ≈500 nucleotides and labeled with ^{32}P by using [γ-^{32}P]ATP and polynucleotide kinase for use as a probe. Cytoplasmic poly(A)$^+$RNA for RNA blots was prepared from IR731 as described (18).

BALB/c $\mu^+\delta^+$ Lymphomas. These lymphomas, the generous gifts of R. Asofsky and K. Jin Kim, were passaged subcutaneously in BALB/c mice (19). The presence of cell-surface IgM and IgD molecules was verified by immunofluorescence using anti-IgM (Cappel) and monoclonal anti-IgD (Becton Dickinson) reagents. GCL-2.1 cells were from W. Raschke. DNA from these and other tissues was prepared by the method of Blin and Stafford (20).

Germ-Line C_δ Clones. The germ-line clone ChSpμ7 has been described (4, 21). The clone ChSp37 was isolated from the same library of mouse sperm DNA as ChSpμ7. The positions of C_δ sequences in these clones were determined by hybridization of [5′-^{32}P]poly(A)$^+$RNA from IR731 to blots of restriction digests of these clones. Subclones of the hybridizing re-

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Abbreviations: V_H, heavy chain variable; C_H, heavy chain constant; kb, kilobase(s).

gions were generated by ligation of restriction fragments into the corresponding site or sites of pBR322 and were used as probes in Southern (22) and RNA blot experiments. The cDNA clone p104Eμ12 (21) was used as a probe for C_μ sequences. The J_H probe, containing the J_H gene cluster and 3'-flanking sequence, was prepared by M. Steinmetz.

BALB/c $\mu^+\delta^+$ Normal B-Cell DNA Library. IgM-positive cells were isolated from BALB/c spleens by using a fluorescence-activated cell sorter. Ninety-three to 97% of the purified cell population stained positively for surface IgM, and 99% was positive for surface IgD. A library of 12×10^6 recombinant phage was constructed by ligation of *Eco*RI partial digests of $\mu^+\delta^+$ spleen cell DNA to phage vector Charon 4A (23), followed by *in vitro* packaging (24).

All manipulations of microorganisms containing recombinant DNA were carried out under P2/EK2 or P2/EK1 conditions prior to January 1980, after which P1/EK2 and P1/EK1 conditions were used.

DNA sequence analysis was as described (25).

RESULTS AND DISCUSSION

Characterization of Genomic Clones. [5'-^{32}P]Poly(A)$^+$RNA prepared from IR731 was used to screen a number of genomic clones known to contain mouse immunoglobulin C_H region genes or their flanking sequences. Two BALB/c sperm DNA clones, ChSpμ7 and ChSp37, hybridized to this probe. These clones were subjected to restriction map analysis (Fig. 1). The [5'-^{32}P]poly(A)$^+$RNA probe hybridized to three discrete regions of the cloned DNA.

Identification of the C_δ Gene. Restriction fragments hybridizing to IR731 poly(A)$^+$RNA were subcloned into the plasmid vector pBR322 (Fig. 1). A DNA sequence determined near the 3' end of the pδ24 clone (Fig. 2, part B) appeared to encode the first third of an immunoglobulin domain, with a cysteine residue and several conserved amino acids in the appropriate positions. This amino acid sequence is translated from the only open reading frame and is associated with a possible downstream splicing site. This sequence displays striking homology to the protein sequence of the C_δ3 domain of human IgD (26): 18 of 33 residues, including a stretch of 10 surrounding the

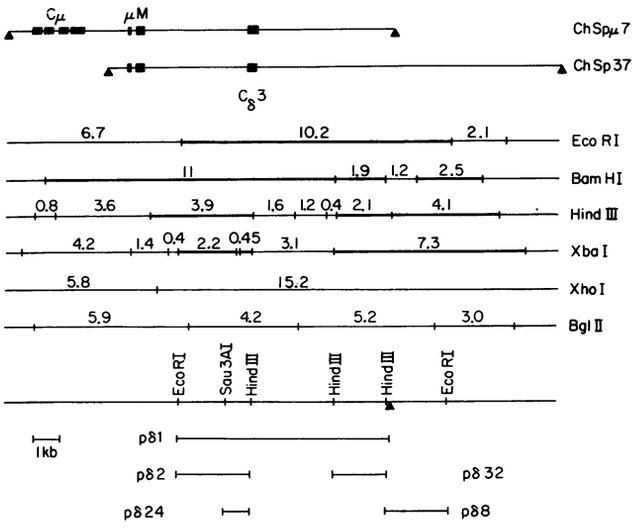

FIG. 1. Restriction map of ChSpμ7 and ChSp37. Restriction fragments of a particular digest that hybridize to [5'-^{32}P]poly(A)$^+$RNA from IR731 are indicated by heavy lines. *Bgl* II and *Xho* I digests were not tested for hybridization to IR731 RNA. Plasmid subclones containing hybridizing regions are also indicated. μM, IgM membrane exons; ▲, synthetic *Eco*RI linker site.

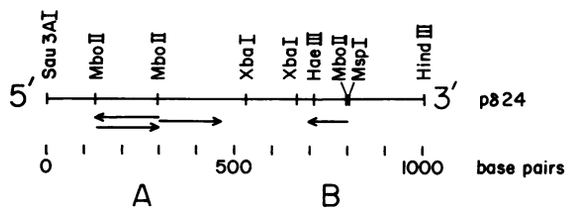

FIG. 2. Partial restriction map of pδ24 and strategy for determination of nucleotide sequence.

cysteine residue are identical, and an additional 10 are either conservative substitutions or can be attributed to a single-base difference in the genetic code. Thus, this sequence codes for the C_δ3 domain of mouse IgD.

Additional sequence determinations in the 5' region of pδ24 (Fig. 2, part A) revealed no additional immunoglobulin domain coding sequences, and the unsequenced portion (coordinates 470–700) is too small to encode a complete immunoglobulin domain. Additional domain and hinge regions presumably lie in pδ2 because no hybridization of IR731 RNA was observed 5' to the 10.2-kb *Eco*RI fragment (Fig. 1). We conclude that in the germ-line the C_δ gene is separated from the C_μ M exons by >2 kb.

C_δ Gene Encodes Two mRNAs with Alternative 3' Ends. The germ-line DNA subcloned in pδ2 contains the C_δ3 domain of the δ gene and, as argued above, probably additional C_δ domains as well. Labeled total cellular poly(A)$^+$RNA from IR731 also hybridized with sequences 3–7 kb to the C_δ3 encoding sequence (Fig. 1). These regions were subcloned as pδ32 and pδ8. Both pδ32 and pδ8 contain only single-copy sequences and do not cross hybridize with each other or with pδ2. That this DNA also contains δ gene sequences was demonstrated by RNA blots with IR731 mRNA and nick-translated pδ32 and pδ8 as probes. Hybridization of C_δ probes (pδ2) to IR731 poly(A)$^+$mRNA revealed a major δ mRNA species of 1.6 kb and a minor species of 2.7 kb (Fig. 3). In addition, the 1.6-kb species hybridized with pδ32, and the 2.7-kb mRNA hybridized with pδ8. These results suggest that pδ32 and pδ8 contain separate, noncontiguous gene segments which represent alternative 3' ends for the 1.6- and 2.7-kb mRNAs. At present we do not know whether each gene sequence is composed of one or more exons. Nonetheless, these results indicate that C_δ gene sequences that are contiguous in δ mRNA occupy about 8 kb of germ-line DNA.

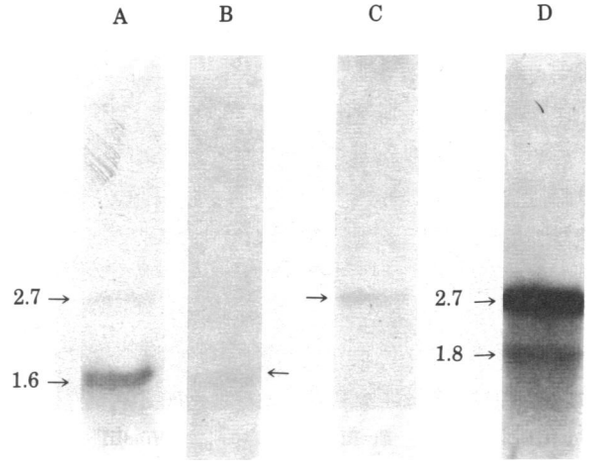

FIG. 3. RNA blots of cytoplasmic mRNA from IR731 rat myeloma (lanes A–C) and GCL-2.1 (lane D), a $\mu^+\delta^+$ mouse cell line, with nick-translated C_δ probes. Lanes: A, pδ2; B, pδ32; C, pδ8; Dpδ2. Fragment sizes are shown in kb.

We have also observed two corresponding species in a mouse $\mu^+\delta^+$ cell line (GCL-2.1) (Fig. 3).

Because the 1.6-kb mRNA is the major species in IR731 myeloma cells which secrete IgD, we propose that pδ32 encodes a δ_s terminus for δ chain secretion. The pδ8 sequence in the 2.7-kb mRNA may encode a δ_m terminus for membrane-bound δ chains. We propose that these alternative 3′ sequences are spliced to the $C_\delta 3$ domain to generate either δ_s or δ_m mRNA. This arrangement is different from that of μ_s and μ_m mRNA, in which the μ_s terminus encoding sequence is contiguous with the $C_\mu 4$ domain (15, 18).

$\mu^+\delta^+$ B-Cell DNA Contains Rearranged C_μ and Germ-Line C_δ Genes. The lymphoma lines L10A and K46 are B-cell tumors and express both IgM and IgD molecules on the cell surface (19). We carried out Southern blot experiments with DNA prepared from these tumor cells, using BALB/c embryo DNA as a control. Fig. 4*A* shows a Southern blot of a *Kpn* I digest of mouse embryo and K46 DNA with the C_μ cDNA clone as probe. In embryo DNA, this C_μ probe hybridizes to a fragment ≈13 kb long. This fragment contains both the C_μ gene and the J_H gene cluster (27). In K46 DNA, the C_μ gene appears on an ≈20-kb restriction fragment, which indicates that a DNA rearrangement has occurred, presumably the V-J joining event (27) (similar results were obtained with L10A DNA). Note that a faint band is present in K46 DNA which corresponds to the germ-line C_μ gene. Both K46 and L10A are polyploid (28), and this band could represent an unrearranged chromosome. Alternatively, it could arise from DNA derived from contaminating host tissue.

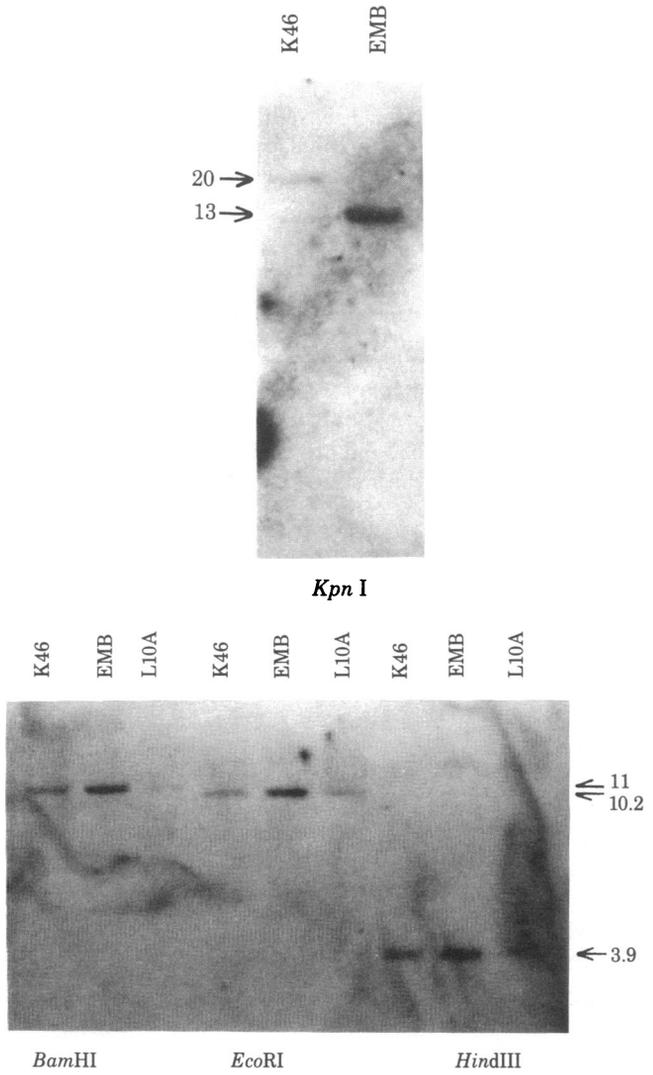

FIG. 4. (*Upper*) Hybridization of p104Eμ12 to *Kpn* I digests of K46 and mouse embryo DNA. (*Lower*) Hybridization of the $C_\delta 3$ probe to *Bam*HI, *Eco*RI, and *Hin*dIII digests of K46, mouse embryo, and L10A DNA. Hybridization conditions: 5X SET, 2X Denhardt's, 0.5% $NaDodSO_4$, 68°C.

The corresponding data for the C_δ gene are shown in Fig. 4*B* for K46, embryo, and L10A DNAs. The probe used in this experiment was a 0.68-kb *Bam*HI/*Xba* I restriction fragment from pδ2 which contains the $C_\delta 3$ domain and 340 nucleotides of pBR322. This C_δ probe hybridizes to identical bands in both embryo and lymphoma DNA restriction digests. No other hybridization could be detected. Furthermore, the bands observed in Fig. 4*B* are identical in size to those observed in ChSpμ7 and ChSp37, which demonstrates that these latter clones contain no detectable cloning artifacts.

Analogous experiments were carried out with pδ8 and pδ32 as probes. In each case, hybridization to germ-line and lymphoma DNA restriction digests gave similar results (data not shown), indicating that no rearrangement of the C_δ gene or its flanking sequences occurs in $\mu^+\delta^+$ lymphoma DNA.

We also have obtained similar results by using the $\mu^+\delta^+$ normal B-cell library. When 1.75×10^6 and 3.2×10^6 recombinant phage were screened with the C_δ and C_μ probes, respectively, a total of 4 C_δ clones and 11 C_μ clones were isolated. Six of the 11 C_μ clones also contained the J_H locus, and 5 of these showed rearrangements consistent with V_H-D-J_H joining. Restriction map analysis of the C_δ and C_μ clones revealed that they all contain only germ-line DNA within the C_μ gene, between C_μ and the downstream *Eco*RI site (Fig. 1), and in the C_δ gene and 3′-flanking sequence. The possibility that we simply failed to clone or detect a rearranged C_δ gene cannot be ruled out; however, these results are consistent with those described above and suggest that, insofar as can be detected by gel electrophoresis, the C_δ gene in $\mu^+\delta^+$ B cell and lymphoma DNA is unrearranged.

Thus, we conclude that the simultaneous expression of IgM and IgD molecules on the B lymphocyte surface does not involve rearrangement of the C_δ gene or, by implication, a V_H-C_δ DNA switch recombination.

IR731 Myeloma DNA Contains a Rearranged C_δ Gene and Has Deleted the C_μ Gene. Using the μ cDNA clone and the restriction fragment encoding the $C_\delta 3$ domain as probes, we also examined the disposition of the C_μ and C_δ genes in IR731 myeloma DNA. DNA from Lou/M/Wsl N rat liver served as a control (Fig. 5). Both probes cross hybridized with the rat genes on a Southern blot of Lou/M/Wsl N liver DNA. Results obtained with the C_μ probe indicate that the C_μ gene has been largely deleted from the myeloma DNA (Fig. 5*A*). The corresponding experiment with the $C_\delta 3$ probe and three restriction endonucleases revealed that the C_δ gene had undergone DNA rearrangement.

These data indicate that the C_δ gene in this IgD-producing myeloma has been rearranged, unlike that in mouse $\mu^+\delta^+$ B cells. Several explanations for these contrasting results should be considered. First, IgD expression in the rat may be fundamentally different from that in the mouse, although we believe that there exists no other evidence to indicate that this might be the case. Second, these observations could be an artifact of the myeloma condition. For example, the significance of "abortive" rearrangements observed in mouse plasmacytomas (29) is not understood. Finally, these results could conceivably be attributed to a minor substrain difference between Lou/M/Wsl N, the tumor host, and Lou/C/Wsl (16), in which IR731 appeared.

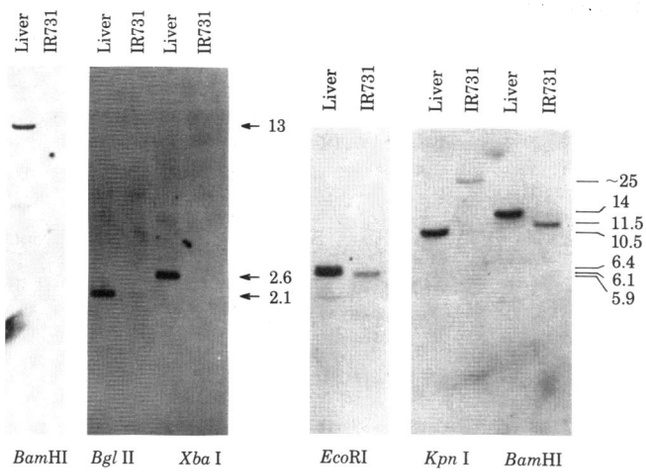

FIG. 5. (*Left*) Hybridization of p104Eμ12 to Lou/M/Wsl N liver and IR731 DNA digested with *Bam*HI, *Bgl* II, and *Xba* I. (*Right*) Southern blot of Lou/M/Wsl N liver and IR731 DNA restriction enzyme digests with the C_δ3 probe. (*C*) Hybridization of the J_H probe to *Hin*dIII and *Bam*HI digests of Lou/MN liver and IR731 DNA. Hybridization conditions were: 5X SET, 2X Denhardt's, 0.5% $NaDodSO_4$, 62°C. Fragment sizes are in kb.

To address the latter two questions, we carried out Southern blot experiments using pδ8, pδ32, a γ3 cDNA clone from myeloma J606, and a plasmid subclone containing the mouse J_H gene cluster and its 3′ flanking sequence. The pδ8 and pδ32 clones gave similar results with Lou/M/Wsl N liver and Lou/C/Wsl myeloma (IR731) DNA: in each case, both hybridized to a single 5.7-kb *Eco*RI fragment. The γ3 cDNA probe also hybridized to 9.2-, 14-, and 16-kb *Eco*RI fragments in both DNA samples. Thus, the C_γ genes and the counterparts of pδ32 and pδ8 in the rat genome display no polymorphism in the two substrains. These results suggest that DNA rearrangement rather than restriction enzyme site polymorphism is responsible for the observed difference in hybridization of the C_δ3 probe to Lou/M/Wsl N liver and IR731 DNA and that this rearrangement has occurred on the 5′ side of pδ32 and pδ8.

Although the C_μ gene had been deleted from IR731 DNA, results obtained with the J_H probe (Fig. 5*C*) indicate that the J_H genes and 3′-flanking sequence are still present and have been rearranged, as would be expected for a *V-D-*J_H joining event. This observation suggests that a V_H–C_δ switch recombination has indeed occurred in IR731 DNA.

Two Alternative Mechanisms for the Expression of IgD. Based on the results described above, we propose the existence of two different molecular mechanisms for the expression of the C_δ gene. First, in $\mu^+\delta^+$ B cells, these results suggest regulation at the level of RNA processing, probably involving multiple sites for splicing and poly(A) addition. This type of control has been observed in late adenovirus mRNA processing (30–32) and has been implicated in the synthesis of membrane-bound and secreted IgM (μ_m and μ_s) mRNA from a single transcript (15). This mechanism presupposes the existence of a poly(A) addition site 3′ to the C_δ gene. Extension of a transcript beyond the poly(A) addition sites of μ_s and μ_m mRNA would then generate the precursor of δ mRNA. Wall *et al.* (33) pointed out that, according to this model, the preferential utilization of the μ_s poly(A) site which would accompany the initiation of μ_s synthesis would halt production of membrane-bound IgM and IgD molecules. Results have been obtained which agree with this prediction (34–36). The C_δ gene thus could be another example in eukaryotes in which developmentally regulated RNA splicing generates alternative protein forms. We believe that this control mechanism will be a general one in eukaryotic gene expression.

The second mechanism, apparently used by the rat IgD myeloma IR731, involves deletion of C_μ and rearrangement of C_δ, presumably via a V_H–C_δ switch recombination. We infer the presence of one or more switch sites (37), probably in the region between C_δ and the IgM M exons. At present, the mechanism of this particular switching event remains unknown. We predict that, generally, IgD-secreting cells also will exhibit rearrangement of the C_δ gene. A schematic representation of these mechanisms is given in Fig. 6.

C_H Gene Linkage Family and C_H Gene Expression. The data in this paper and those from other laboratories (38–40), allow construction of a linkage map of the immunoglobulin heavy chain gene family (Fig. 6, top line). Because the separation between the C_μ and C_δ genes is substantially smaller than that for other C_H genes, we believe that the C_μ–C_δ system is likely to be the only pair of C_H genes that uses an RNA processing mechanism as proposed above for their expression. Expression of other heavy chain genes (C_γ, C_α, C_ϵ) most likely will occur only by the C_H switching mechanism involving DNA rearrangement.

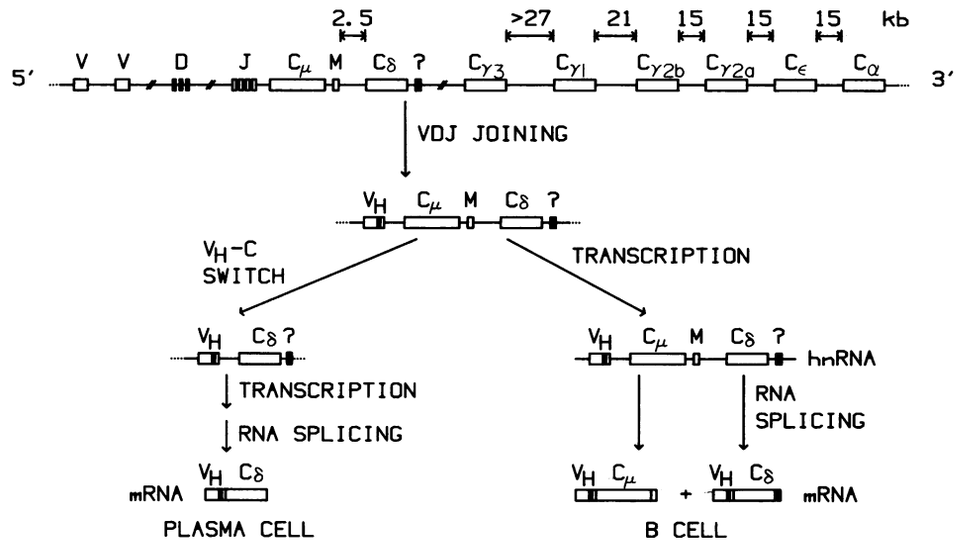

FIG. 6. Proposed mechanisms for expression of IgD in B cells and plasma cells. The top line represents the current germ-line linkage map (38–40) of the immunoglobulin C_H locus. Known distances are in kb. The putative δ_m exon is indicated by ?.

Note. After this manuscript was submitted for review, two articles (41, 42) describing detailed structural studies of the mouse C_δ gene appeared. Our data on the structure of the C_δ gene largely agree with the data presented in them.

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