Correction. In the article "Flow of information in the light-triggered cyclic nucleotide cascade of vision" by Bernard K.-K. Fung, James B. Hurley, and Lubert Stryer, which appeared in the January 1981 issue of *Proc. Natl. Acad. Sci. USA* (78, 152-156), an undetected printer's error occurred on p. 155. In the first paragraph of the Discussion, the fifth sentence should read as follows: "The first reaction can occur in the absence of phosphodiesterase and the second can take place in the absence of photolyzed rhodopsin."

Correction. In the article "Expression of IgD may use both DNA rearrangement and RNA splicing mechanisms" by K. W. Moore, J. Rogers, T. Hunkapiller, P. Early, C. Nottenburg, I. Weissman, H. Bazin, R. Wall, and L. E. Hood, which appeared in the March 1981 issue of *Proc. Natl. Acad. Sci. USA* (78, 1800-1804), part C of Fig. 5 was omitted. It and its legend appear below.

![HindIII and BamHI digest of Lou/MN liver and IR731 DNA](image)

**FIG. 5C.** Hybridization of the JH probe to HindIII and BamHI digesta of Lou/MN liver and IR731 DNA. Hybridization conditions were: 5x SET, 2x Denhardt's, 0.5% NaDodSO4, 62°C. Fragment sizes are in kb.
Expression of IgD may use both DNA rearrangement and RNA splicing mechanisms

K. W. Moore*, J. Rogers†, T. Hunkapiller*, P. Early*, C. Nottenburg†, I. Weissman‡, H. Bazin§, R. Wall†, and L. E. Hood*

*Division of Biology, California Institute of Technology, Pasadena, California 91125, †Molecular Biology Institute and Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, California 90024, ‡Laboratory of Experimental Oncology, Department of Pathology, Stanford Medical School, Stanford, California 94305, and †Experimental Immunology Unit, University of Louvain, Brussels, Belgium.

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ABSTRACT From a library of mouse sperm DNA, we have isolated two overlapping clones which contain the C\textsubscript{\textmu} gene. One of these clones also contains the C\textsubscript{\sigma} gene. The C\textsubscript{\sigma} gene is separated from the C\textsubscript{\textmu} membrane exons by approximately 2 kilobases (kb) of DNA. The C\textsubscript{\textmu} gene was identified by (a) hybridization to poly(A\textsuperscript{+}) RNA prepared from the Ig\textmu-producing rat plasma cell tumor IR731, and (b) homology of a translated nucleotide sequence to the amino acid sequence of the human \textgreek{b} chain. The C\textsubscript{\textmu} gene spans 8 kb of DNA in the germ line. Plasmid subclones of the C\textsubscript{\sigma} gene were used as probes in Southern and RNA blot experiments. RNA blot analysis of cytoplasmic poly(A\textsuperscript{+}) RNA from IR731 and a \mu\textsuperscript{+}\delta\textsuperscript{-} B-cell hybridoma revealed 1.6- and 2.7-kb \delta mRNA species with different 3' ends, which presumably encode the secreted and membrane-bound forms, respectively, of the \delta chain. Southern blot analysis of DNA from two \mu\textsuperscript{+}\delta\textsuperscript{-} lymphomas revealed that the C\textsubscript{\delta} gene is in the germ-line configuration in each case. Restriction map analysis of C\textsubscript{\delta} and C\textsubscript{\textmu} genomic clones isolated from a library of normal \mu\textsuperscript{+}\delta\textsuperscript{-} B-cell DNA also gave no evidence for DNA rearrangement in the region between the C\textsubscript{\textmu} and C\textsubscript{\delta} genes. Taken together, these data suggest that IgD expression in \mu\textsuperscript{+}\delta\textsuperscript{-} B cells does not involve a V\textsubscript{H}-to-C\textsubscript{\textmu} DNA switch rearrangement. We propose that simultaneous expression of C\textsubscript{\textmu} and C\textsubscript{\delta} with a single V\textsubscript{H} gene is mediated by two alternative routes of RNA processing of a primary nuclear transcript which contains the V\textsubscript{H}, C\textsubscript{\textmu}, and C\textsubscript{\delta} genes. In contrast, analogous experiments with myeloma IR731 DNA revealed that the C\textsubscript{\mu} gene has been deleted from the myeloma DNA and that the C\textsubscript{\delta} gene has undergone DNA rearrangement, presumably including a switch recombination of the V\textsubscript{\delta} gene from the C\textsubscript{\textmu} to the C\textsubscript{\delta} 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\textsubscript{H}) region and one of five classes of constant (C\textsubscript{H}) region: C\textsubscript{\textmu} (IgM), C\textsubscript{\sigma} (IgD), C\textsubscript{\alpha} (IgG), C\textsubscript{\gamma} (IgA), and C\textsubscript{\delta} (IgE). In mice, the five C\textsubscript{H} classes are encoded by eight distinct genes: C\textsubscript{\textmu}, C\textsubscript{\sigma}, C\textsubscript{\alpha}, C\textsubscript{\gamma}, C\textsubscript{\delta}, C\textsubscript{\gamma}, and C\textsubscript{\delta}. 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\textsubscript{H} gene is joined to the C\textsubscript{\textmu} gene by assembly of three gene segments: V, D, and J (2, 3). Presumably, upon recognition of antigen, the V\textsubscript{H} gene, along with some C\textsubscript{\textmu} 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\textsubscript{\textmu} gene and perhaps other C\textsubscript{\sigma} genes, is deleted.

Both IgM and IgD molecules are present on the surface of the B cell. Experiments utilizing alleotypic 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\textsubscript{H} regions (10–13). These data are difficult to reconcile with the C\textsubscript{\textmu} gene deletion model because they imply that a V\textsubscript{H} gene can be expressed with the C\textsubscript{\sigma} gene without concomitant deletion of the C\textsubscript{\textmu} 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\textsubscript{H} gene is joined to the C\textsubscript{\sigma} gene while the original remains joined to the C\textsubscript{\textmu} gene, and differential RNA processing of a single transcript containing V\textsubscript{H}, C\textsubscript{\textmu}, and C\textsubscript{\sigma} genes (15).

This paper reports experiments carried out to test these hypotheses. The results support RNA splicing as the mechanism by which the C\textsubscript{\textmu} and C\textsubscript{\sigma} genes are expressed simultaneously in B cells and also suggest that V\textsubscript{H}-to-C\textsubscript{\textmu} 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/Ws\textsuperscript{N} rats (NIH). Total cell poly(A\textsuperscript{+}) RNA was prepared from IR731 by a method similar to that of Chirgwin et al. (17), followed by oligo(T)-cellulose chromatography. This RNA was hydrolyzed with base to an estimated average size of ~500 nucleotides and labeled with \textsuperscript{32}P by using [\gamma\textsuperscript{32}P]ATP and polynucleotide kinase for use as a probe. Cytoplasmic poly(A\textsuperscript{+}) RNA for RNA blots was prepared from IR731 as described (18).

BALB/c \mu\textsuperscript{+}\delta\textsuperscript{-} 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\textsubscript{\sigma} Clones. The germ-line clone ChSp\mu\textmu7 has been described (4, 21). The clone ChSp37 was isolated from the same library of mouse sperm DNA as ChSp\mu\textmu7. The positions of C\textsubscript{\sigma} sequences in these clones were determined by hybridization of \textsuperscript{5'}\textsuperscript{32}Ppoly(A\textsuperscript{+}) RNA from IR731 to blots of restriction digests of these clones. Subclones of the hybridizing re-

Abbreviations: V\textsubscript{H}, heavy chain variable; C\textsubscript{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 p104EµI2 (21) was used as a probe for C<sub>µ</sub> sequences. The J<sub>H</sub> probe, containing the J<sub>H</sub> gene cluster and 3'-flanking sequence, was prepared by M. Steinmetz.

BALB/c µ<sup>-5</sup> 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 90% was positive for surface IgD. A library of 12 × 10<sup>6</sup> recombinant phage was constructed by ligation of EcoRI partial digests of µ<sup>-5</sup> 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',32P]Poly(A)<sup>+</sup>RNA prepared from IR731 was used to screen a number of genomic clones known to contain mouse immunoglobulin C<sub>H</sub> region genes or their flanking sequences. Two BALB/c spermatid DNA clones, ChSp<sub>µ</sub>7 and ChSp<sub>µ</sub>7, hybridized to this probe. These clones were subjected to restriction map analysis (Fig. 1). The [5',32P]poly(A)<sup>+</sup>RNA probe hybridized to three discrete regions of the cloned DNA.

Identification of the C<sub>µ</sub> Gene. Restriction fragments hybridizing to IR731 poly(A)<sup>+</sup>RNA were subcloned into the plasmid vector pBR322 (Fig. 1). A DNA sequence determined near the 3' end of the p824 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<sub>µ</sub>3 domain of human IgD (26): 18 of 33 residues, including a stretch of 10 surrounding the

![Fig. 1. Restriction map of ChSp<sub>µ</sub>7 and ChSp<sub>µ</sub>37. Restriction fragments of a particular digest that hybridize to [5',32P]poly(A)<sup>+</sup>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; a, synthetic EcoRI linker site.](image1)

![Fig. 2. Partial restriction map of p824 and strategy for determination of nucleotide sequence.](image2)

![Fig. 3. RNA blots of cytoplasmic mRNA from IR731 rat myeloma (lanes A–C) and GCL-2.1 (lane D), a µ<sup>-5</sup> mouse cell line, with nick-translated C<sub>µ</sub> probes. Lanes: A, p<sub>82</sub>; B, p<sub>832</sub>; C, p<sub>68</sub>; Dp<sub>82</sub>. Fragment sizes are shown in kb.](image3)
We have also observed two corresponding species in a mouse \( \mu^\ast \delta^\ast \) 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 p88 encodes a \( \delta \) terminus for \( \delta \) chain secretion. The p88 sequence in the 2.7-kb mRNA may encode a \( \delta_m \) terminus for membrane-bound \( \delta \) chains. We propose that these alternative 3' sequences are spliced to the \( C_\mu \beta \) domain to generate either \( \delta \) or \( \delta_m \) mRNA. This arrangement is different from that of \( \mu \) and \( \mu_m \) mRNA, in which the \( \mu_m \) terminus encoding sequence is contiguous with the \( C_\mu \) domain (15, 18).

**\( \mu^\ast \delta^\ast \) B-Cell DNA Contains Rearranged \( C_\mu \) and Germ-Line \( C_\delta \) 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 gene cluster (27). In K46 DNA, the \( \mu \) gene cluster contains both the \( C_\mu \) gene and the \( J_H \) gene cluster (27). In K46 DNA, the \( C_\mu \) gene appears on an

\[ \text{Fig. 4. (Upper) Hybridization of p104E} \mu12 \text{ to Kpn I digests of K46 and mouse embryo DNA. (Lower) Hybridization of the C}_\beta \text{ probe to BamHI, EcoRI, and HindIII digests of K46, mouse embryo, and L10A DNA. Hybridization conditions: 5X SET, 2X Denhardt's, 0.5\% NaDodSO}_4, 68^\circ \text{C}.} \]
J606, clones gave gene of the restriction to blot conditions. Thus, sizes occurred. The results of the C83 probe were used. We observed hybridization of the C83 probe to HindIII and BamHI digests of the Lou/M liver and IR731 DNA. Hybridization conditions were: 5X SET, 2% Denhardt's, 0.5% NaDodSO₄, 62°C. Fragment sizes are in kb.

To address the latter two questions, we carried out Southern blot experiments using p88, p382, a J3 cDNA clone from myeloma J606, and a plasmid subclone containing the mouse JH gene cluster and its 3' flanking sequence. The p88 and p382 clones gave similar results with Lou/M/WS1 N liver and Lou/M/WS1 myeloma (IR731 DNA): in each case, both hybridized to a single 5.7-kb EcoRI fragment. The J3 cDNA probe also hybridized to 9.2-, 14-, and 16-kb EcoRI fragments in both DNA samples. Thus, the Cg genes and the counterparts of p382 and p88 in the rat genome display no polymorphism in the two sub-strains. These results suggest that DNA rearrangement rather than restriction enzyme site polymorphism is responsible for the observed difference in hybridization of the C83 probe to Lou/M/WS1 N liver and IR731 DNA and that this rearrangement has occurred on the 5' side of p382 and p88. Although the Cg gene had been deleted from IR731 DNA, results obtained with the Jg probe (Fig. 5C) indicate that the JH genes and 3'-flanking sequence are still present and have been rearranged, as would be expected for a V-D-JH joining event. This observation suggests that a Vμ-Cg 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 Cg gene. First, in μδ 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 (μm and μ) mRNA from a single transcript (15). This mechanism presupposes the existence of a poly(A) addition site 3' to the Cg gene. Extension of a transcript beyond the poly(A) addition sites of μ and μm 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, poly(A) site which would accompany the initiation of δ synthesis would halt production of membrane-bound IgM and IgD molecules. Results have been obtained which agree with this prediction (34–36). The Cg 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 Cg and rearrangement of Cg, presumably via a Vg-Cg switch recombination. We infer the presence of one or more switch sites (37), probably in the region between Cg 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 Cg gene. A schematic representation of these mechanisms is given in Fig. 6.

Cg Gene Linkage Family and Cg 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 Cg and Cg genes is substantially smaller than that for other Cg genes, we believe that the Cg-Cg system is likely to be the only pair of Cg genes that uses an RNA processing mechanism as proposed above for their expression. Expression of other heavy chain genes (Cg, Cg, Cg) most likely will occur only by the Cg switching mechanism involving DNA rearrangement.

![Fig. 6](image_url) 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 Cg locus. Known distances are in kb. The putative δ exon is indicated by ?.
Note. After this manuscript was submitted for review, two articles (41, 42) describing detailed structural studies of the mouse C \(_5\) gene appeared. Our data on the structure of the C \(_5\) gene largely agree with the data presented in them.

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