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Independent Immunoglobulin Class-Switch Events Occurring in a Single Myeloma Cell Line

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Five γ2a-producing cell lines, all derived from the γ2b-producing mouse myeloma MPC11, were analyzed for changes in gene structure. Also examined was a cell line (ICR9.7.1) that acts as an intermediate to some of these class switches. All six of the MPC11 variants have undergone DNA rearrangement. Rearrangement sites in the γ2a-producing cells are different in each case and generally do not involve tandem repeat sequences. An enhancer for heavy chain gene transcription was deleted in at least one of these cell lines as a result of its class-switch rearrangement. DNA sequence analysis of cloned genes from two of the MPC11 variants revealed shared sequences at their rearrangement breakpoints. The same sequences are found at the breakpoints of two additional immunoglobulin gene rearrangements in MPC11.

The antigen-binding specificity of a B cell becomes established through a rearrangement of immunoglobulin gene segments (V-J and V-D-J joining). An antibody-producing cell can maintain this antigen specificity while changing the class of heavy chain it produces through a second gene rearrangement event. This process is referred to as heavy chain class switching. The different heavy chain classes, which are distinguished by their constant region (C_H) sequences, confer different biological activities on the assembled immunoglobulin molecule, e.g., ability to pass through the placenta, to fix complement, and to facilitate phagocytosis through binding to class-specific Fc receptors on macrophages.

The mechanism for heavy chain class-switch rearrangements and the means by which this process is initiated in the antibody-producing cell have yet to be identified. However, we (10) and others (32) have shown that gene rearrangements resulting in a heavy chain class switch can occur in cultured myeloma cells.

We have been examining class switches that take place in culture to ask how they might illuminate the process of heavy chain class switching as it occurs in animals. The system that we have been studying consists of a parental γ2b, K-producing cell line (MPC11), three γ2a, K-producing cell lines (11.8, M319.2, and M224) (29) that were isolated directly from mutagen-treated MPC11 cells, an intermediate variant cell line (9.7.1) (29) that also arose from mutagenized MPC11 cells and that produces an abnormally large heavy chain, and two γ2a, K-producing secondary variant cell lines (9.9.2.1 and 9.9.1.6.7) (15) that arose spontaneously from cell line 9.7.1 (Fig. 1). Most of these class-switch variants, then, were isolated from mutagenized MPC11 cells. However, similar class-switch variants arise spontaneously in untreated MPC11 cell cultures and in other myeloma and hybridoma cell cultures (9, 18, 24, 30, 37a, 39).

All five of the MPC11-derived γ2a-producing cell lines make normal-sized heavy chains, and they, along with 9.7.1, were shown in earlier studies (11) to retain variable region (V_H) determinants in common with the MPC11 parent. In fact, it has recently been shown through DNA sequence analysis that the expressed V_H coding sequences in two of the variants, 9.7.1 and 9.9.2.1, are identical to each other and to that of MPC11 (G. L. Gilmore and B. K. Birshtein, unpublished data). At the level of immunoglobulin phenotype, then, these variants represent the products of a heavy chain class switch (from the γ2b to the γ2a class) similar to that which occurs during normal B-lymphocyte differentiation. Class switches from μ to the other heavy chain classes have been observed in mitogen-stimulated cultures of normal B lymphocytes and inferred from developmental studies in the mouse. The experimental materials available for these studies generally do not allow for the detection of subsequent switches among the other (non-μ) classes (only surface immunoglobulin M [IgM]-positive and surface IgA-positive B-cell populations can be obtained in sufficient numbers for analysis). However, the immunoglobulin gene structure in some myelomas suggests that B cells (or plasmacytes) sometimes undergo this type of class switch as well (e.g., μ to γ2b to ε; 27, 28).

Our earlier studies (10) of heavy chain gene structure in the MPC11 class-switch variants indicated that each variant had undergone gene rearrangement with respect to the parental (MPC11) cell line. In the present study, the 5′ breakpoints of the rearrangements in each of five γ2a-producing variant cell lines have been localized within the noncoding sequences separating V_{MPC11} and Cγ2b. In two of these cell lines, the 3′ rearrangement breakpoints have also been identified. With one exception, the class-switch rearrangements that have taken place in these cultured myeloma cells have breakpoints that fall considerably outside of tandem repeat sequences flanking Cγ2b and Cγ2a. Deletion of an enhancer for heavy chain gene expression has resulted from the class-switch rearrangement in at least one, and possibly two, of the MPC11 variants. Both variants make normal amounts of heavy chains (11; D. M. Zaller and L. A. Eckhardt, unpublished data).

We determined the DNA sequence surrounding the 5′ and 3′ rearrangement breakpoints in one of the γ2a-producing MPC11 variants (9.9.2.1) and in the class-switch intermediate 9.7.1. A common sequence is found at the breakpoints in both variants. These breakpoint sequences are also present at the breakpoints of two gene rearrangements that occurred in the MPC11 cell lineage before its establishment as a tumor.

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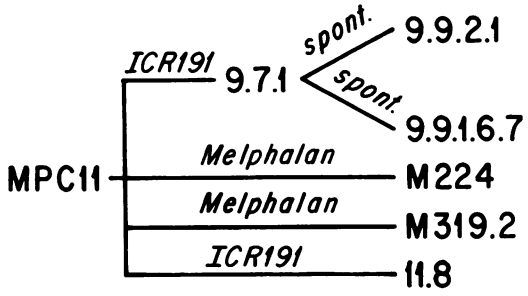


FIG. 1. Derivation of γ2a-producing class-switch variants from the MPC11 cell line.

MATERIALS AND METHODS

Cell lines. 45.6.2.4 is a cloned cell line derived from the MPC11 (γ2b, K) BALB/c tumor cell line (17). The MPC11 DNA used in these studies was isolated from that cell line. 9.7.1 is a variant cell line isolated after mutagen (ICR191) treatment of 45.6.2.4 cells (29). It produces a 75,000-molecular-weight (MW) heavy chain that is not secreted and spontaneously gives rise to secondary variants (e.g., 9.9.2.1 and 9.9.1.6.7) that produce and secrete γ2a heavy chains of normal (MW 55,000) size (15). M224, M319.2, and 11.8 are γ2a-producing cell lines that were isolated directly from 45.6.2.4 cells after mutagen (melphalan or, for 11.8, ICR191) treatment (29).

Agarose gel electrophoresis and transfer to nitrocellulose. DNA was prepared from cultured myeloma cell lines essentially by the method of Walker and McLaren (40). Enzyme-digested myeloma DNA (7 to 10 μg) was loaded in each track of a 0.8% agarose gel (buffer, 0.089 M Tris-hydrochloride [pH 8.3]–0.023 M H_3PO_4–2.5 mM EDTA). After electrophoresis, DNA was transferred from the gels to nitrocellulose by the method of Southern (36). For mapping of recombinant phage DNA, ~0.25 μg of enzyme-digested DNA was loaded per gel track, and after electrophoresis, it was transferred to a Gene Screen filter (New England Nuclear Corp.).

Construction of myeloma DNA libraries in λ phage. (i) 9.7.1. High-MW 9.7.1 DNA was methylated to completion at the *Eco*RI recognition sites by using *Eco*RI methylase (New England Biolabs, Inc.). After extraction with an equal volume of phenol (saturated with 0.2 M Tris-hydrochloride [pH 8.0]), the methylated DNA was dialyzed against 10 mM Tris-hydrochloride (pH 8.0)–1 mM EDTA (TE buffer). The DNA was then adjusted to 50 μg/ml in 25 mM Tris-hydrochloride (pH 8.5)–2.5 mM $MgCl_2$ and digested at 37°C with 15 U of *Eco*RI restriction enzyme per μg of DNA (conditions required for *Eco*RI* activity). Partially digested DNA was recovered at several time points after the addition of the restriction enzyme, and the reaction was terminated in these samples by the addition of EDTA (final concentration, 20 mM). Small aliquots of the DNA collected at these time points were analyzed by electrophoresis through a 0.6% agarose gel. Fully digested DNA was then pooled with the partially digested DNA, ethanol precipitated, resuspended in TE buffer, and then loaded (100 μg) on a 12.5-ml 10 to 40% sucrose gradient (made in 1 M NaCl–20 mM Tris-hydrochloride [pH 8.0]–2.5 mM EDTA). After spinning for 18 h at 31,000 rpm (Beckman SW41), fractions were collected and analyzed for size on a 0.6% agarose gel. Those fractions with DNA fragments in the size range of 15 to 24 kilobases (kb) were pooled, and the DNA was precipitated with ethanol (yield, 10 μg).

Charon 4 (Ch4) phage (41) was grown in the KH802 strain of *Escherichia coli*, and the phage DNA was isolated essentially as described by F. R. Blattner in the protocol that accompanies the Charon λ phages. Phage DNA was cut to completion with *Eco*RI, and the phage arms were separated from internal *Eco*RI fragments by fractionation on a 10 to 40% sucrose gradient.

Of the 15 to 24-kb methylated and partially *Eco*RI*-digested myeloma DNA, 10 μg was ligated to 16 μg of Ch4 arm DNA (in 40 mM Tris-hydrochloride [pH 7.8]–10 mM $MgCl_2$–20 mM dithiothreitol–1 mM ATP–50 μg of bovine serum albumin per ml) by using T4 DNA ligase (New England Biolabs). Aliquots of this ligated DNA were in vitro packaged with phage packaging extracts prepared essentially by the method of Collins and Hohn (8) from the NS428 and NS433 bacterial strains (38). The packaging efficiency of uncut Ch4 DNA with these extracts was $\sim 2 \times 10^7$ PFU/μg, and the efficiency of the ligated myeloma-Ch4 DNA was between 6×10^5 and 10×10^5 PFU/μg of Ch4 arms. The packaged DNA was used to infect KH802 and plated, for amplification, at ca. 2.5×10^4 PFU per L-agar plate (Nunc; 22 by 22 cm). The resulting plaques were counted to estimate the actual number of phage per plate, the plates were overlaid with 30 ml of phage storage buffer (10 mM Tris-hydrochloride [pH 7.4], 100 mM NaCl, 10 mM $MgCl_2$, 0.05% gelatin) containing a few drops of chloroform and placed at 4°C with rocking for 8 to 16 h. The buffer was then recovered, spun to remove bacterial cells, and stored at 4°C. Appropriately sized aliquots of these amplified pools of recombinant phage were then plated (at a density of 10^5 PFU per plate) on L-agar plates (22 by 22 cm), nitrocellulose filters were lifted, and the filters were hybridized with pJ11 (see below). More than 90% of the phage in the 9.7.1 DNA library were recombinants, as judged by a test for phage with β-galactosidase activity (5). Fourteen plaques, positive with the pJ11 probe, were detected on one screening plate after 7×10^5 recombinant PFUs had been screened. The restriction map of one of these, Ch9.7.1, is shown (see Fig. 3).

(ii) 9.9.2.1. 9.9.2.1 genomic DNA was cut to completion with *Eco*RI and fractionated on a 10 to 40% sucrose gradient. Fractions enriched for DNA fragments between 7 and 8 kb in size were pooled, and the DNA was ethanol precipitated and then ligated with an equal (wt/wt) amount of *Eco*RI-digested Charon 16A (41) DNA. One-third of the phage produced after packaging of this ligated DNA (packaging efficiency, 5×10^5 to 15×10^5 PFU/μg) were recombinants, as judged by tests for β-galactosidase activity. In the latter stages of screening, the phage were not amplified before screening. More than 10^6 PFUs were screened before a pJ11-positive plaque (Ch9.9.2.1) was identified.

Nomenclature. Restriction maps of the immunoglobulin heavy chain genes in MPC11 and variants are drawn 5′ to 3′ with respect to heavy chain gene transcription. Thus, with respect to germ line sequences, variants have 5′ and 3′ rearrangement breakpoints which form the class-switch rearrangement site.

DNA hybridizations. Hybridization probes included pBR1.4, a 1.4-kb mouse liver DNA fragment cloned in pBR322 (16), pJ11, a *Bam*HI-*Eco*RI fragment derived from the J_H gene region of BALB/cJ liver DNA and subcloned into pBR322 (19), and Sγ2a-3, a mouse embryonic *Eco*RI fragment that maps 5′ of Cγ2a. The latter fragment was subcloned into pBR325 from a recombinant phage, ChMγ2a-9 (35), kindly sent to us by T. Honjo. The remaining hybridization probes used in these studies were derived from phage isolated from the myeloma gene libraries described

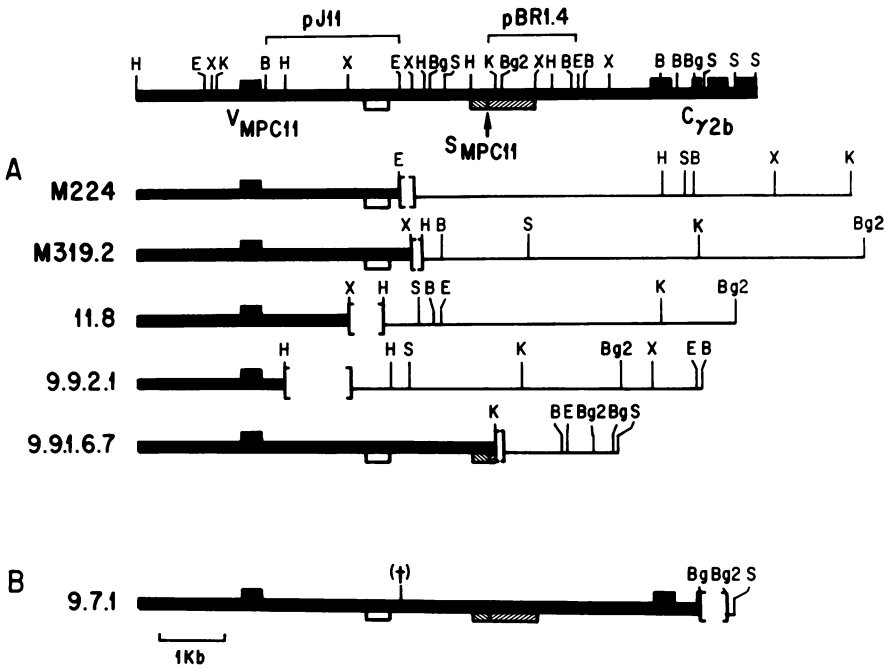


FIG. 2. Rearrangements in the expressed heavy chain genes of six MPC11 variants. A restriction map of the parental (MPC11) γ2b gene is shown on the top line and compared with the heavy chain genes of class-switch variants making γ2a heavy chains (A) and an intermediate variant (9.7.1) making a heavy chain of abnormal size (B). The restriction map of the MPC11 gene was derived from analysis of both total genomic DNA and cloned DNA. The gene maps for the variants are based on analysis of total genomic DNA only. The regions from which pJ11 and pBR1.4, the hybridization probes used for these studies, were derived are indicated above the MPC11 map. pJ11-complementary sequences are found within the expressed heavy chain gene of MPC11 and in no other region of the MPC11 genome (16). Regions of identity between the parental and variant maps are indicated by a thick line, and the restriction sites in these regions are shown only on the MPC11 map. Restriction sites at the point of divergence between parent and variant maps are shown on the variant maps, and the regions enclosed by brackets mark the area of class-switch rearrangement as determined by these comparisons. The open boxes below the gene maps mark the position of a heavy chain gene transcriptional enhancer. The hatched boxes denote areas consisting of tandem repeat sequences as determined by Nikaido et al. (27). The V_{MPC11} and Cγ2b exons are shown as solid boxes above the map. The μ to γ2b class switch, which took place before the isolation of MPC11 as a γ2b-producing tumor, is evidenced by a class-switch rearrangement site (S_{MPC11}) in the MPC11 gene. †, An *Eco*RI site that is present in MPC11 but which has been lost in 9.7.1, most likely as the result of a point mutation. H, *Hin*dIII; E, *Eco*RI; X, *Xba*I; K, *Kpn*I; B, *Bam*HI; Bg, *Bgl*I; Bg2, *Bgl*II; S, *Sst*I.

above. In most cases, pBR325 was the vector used for subcloning phage fragments.

All probes were nick translated (31) to a specific activity of $>10^8$ cpm/μg. Nitrocellulose filters containing myeloma total genomic DNA were hybridized as described (10). Gene Screen (New England Nuclear) filters containing blots of gel-fractionated phage DNA were hybridized for 18 to 24 h at 65°C in 0.75 M NaCl–0.075 M sodium citrate–0.04% polyvinylpyrrolidone–0.04% bovine serum albumin–0.1% Ficoll. Sheared and heat-denatured salmon sperm DNA was present at a concentration of 50 μg/ml, polyadenylic acid was present at a concentration of 20 μg/ml, and ^{32}P-labeled probe was present at 2×10^5 cpm/ml. Filters lifted from phage screening plates were hybridized in essentially the same hybridization mix, except that it was buffered with 50 mM sodium phosphate (pH 7.4) and made 50% with formamide. As many as 10 phage library screening filters (22 by 22 cm) were hybridized in a total volume of 200 ml with 2×10^5 cpm of probe per ml. Duplicate filters were lifted from each screening plate to facilitate the identification of true positives. Both types of phage hybridizations (plaque lifts and gel-fractionated phage DNA) were washed at room temperature in 0.3 M NaCl–0.03 M sodium citrate–0.2% sodium dodecyl sulfate.

DNA sequencing. DNA was sequenced by the method of Maxam and Gilbert (21) or by the dideoxy-chain termination method (22, 33). In the latter cases, DNA fragments of the relevant regions were subcloned into M13 phage (mp8 or mp9) (23).

Sequences of the germ line region spanning the J_H genes (in BALB/c cells) were obtained from GenBank, as were germ line sequences of the BALB/c Cγ2b gene. Initially, we noted many discrepancies between our sequence of the J_H region in 9.9.2.1 and that published by another laboratory. However, we found that with the exception of only a few base pairs (bp), our sequence was identical to the one available for this region in GenBank. The latter represents a compilation of sequence data from several laboratories. We would like to emphasize the usefulness of the GenBank system for resolving discrepancies in individual sequences due to technical error or mutations (arising in cell lines under study or in the process of molecular cloning). Sequences were aligned by using the CompN program (34).

RESULTS

Our earlier studies (10) of the MPC11 class-switch variants suggested that all retained a single copy of the assembled MPC11V_H coding sequences, but that four out of five variants had undergone rearrangement within a 2.0-kb *Hin*dIII restriction fragment mapping 0.4 to 2.4 kb 3′ (with respect to V_H transcription) of these sequences (Fig. 2). The fifth variant (9.9.1.6.7) retained the 2.0-kb *Hin*dIII fragment in common with MPC11, suggesting that its class-switch rearrangement involved sequences farther downstream. Us-

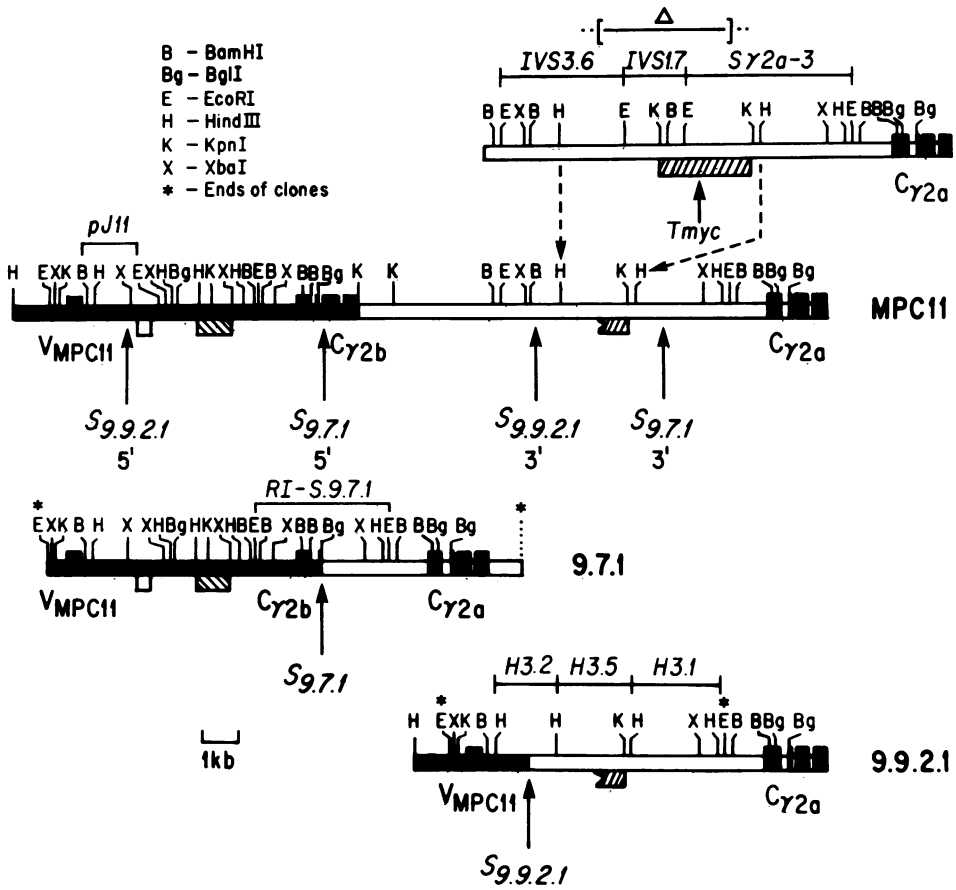


FIG. 3. Structure of the expressed heavy chain genes in 9.7.1 and 9.9.2.1. Restriction maps of the heavy chain genes expressed in these MPC11 variants and cloned in lambda phage are shown and compared with a region of the chromosome from which the MPC11 heavy chain is expressed. Most of the region separating Cγ2b and Cγ2a on the map of MPC11 has been mapped by comparative analysis of total genomic DNA from MPC11 with cloned DNA from the MPC11 variants and mouse germ line. The top line of the figure is a partial map of the sequences flanking Cγ2a as they exist in germ line. A deletion that has taken place within this region on the expressed chromosome of MPC11 is indicated (Δ), and dashed arrows align comparable *Hin*dIII sites on the germ line and MPC11 maps. The exact endpoints of the deletion have not been determined. Subcloned fragments used as hybridization probes or otherwise mentioned in the text are indicated above the germ line and 9.9.2.1 gene maps. The open boxes below the MPC11 and 9.7.1 gene maps mark the position of a heavy chain gene transcriptional enhancer, and hatched boxes on all of the maps mark regions containing tandem repeat sequences. V_{MPC11}, Cγ2b, and Cγ2a exons are shown as closed boxes.. $S_{9.7.1}$ and $S_{9.9.2.1}$ are the sites of class-switch rearrangement in these two variants, and the 5′ and 3′ breakpoints for both rearrangements are indicated on the MPC11 map ($S_{9.9.2.1}$ 5′, etc.). 5′ and 3′ are defined with respect to heavy chain gene transcription. The Ch9.7.1 and Ch9.9.2.1 recombinant phage clones contain the DNA bordered by asterisks on the 9.7.1 and 9.9.2.1 maps, respectively. *Tmyc* marks the site 5′ of Cγ2a, which, on the unexpressed C_H chromosome (chromosome 12) of MPC11, has undergone a reciprocal translocation with c-*myc* (chromosome 15).

ing both the pJ11 and pBR1.4 hybridization probes (Fig. 2), we extended our analysis of genomic DNA from these cell lines to generate the restriction maps shown in Fig. 2. For these mapping studies we made use of the fact that pJ11-complementary sequences are found only within the expressed heavy chain gene of MPC11 and in no other region of the MPC11 genome (16).

Whereas pJ11 covers ~2.0 kb of the intervening sequence between V_{MPC11} and Cγ2b, pBR1.4 detects an additional 1.4 kb of this intervening sequence (Fig. 2). pJ11 mapping data placed the 5′ rearrangement breakpoint in 9.9.1.6.7 within the tandem repeat sequences flanking Cγ2b. As expected then, since pBR1.4 maps in the same region as the tandem repeats, sequences complementary to this probe are retained downstream of V_{MPC11} in 9.9.1.6.7. The maps of M224, 11.8, and 9.9.2.1 diverge from that of the expressed gene of MPC11 5′ of pBR1.4 sequences, and we found that sequences complementary to pBR1.4 were absent from the expressed chromosome in these cell lines. Such sequences were retained in these three variants only in association with the unexpressed copy of Cγ2b. We were able to distinguish restriction fragments derived from regions flanking the expressed and unexpressed copies of Cγ2b, since these differ in their restriction enzyme patterns 5′ of the S_{MPC11} site shown in Fig. 2 (16). Although it was predicted that M319.2 would have similarly lost pBR1.4 sequences from the expressed chromosome, we have found that some of these sequences are retained, suggesting a complex rearrangement in this cell line.

In summary, the switch from γ2b heavy chain production to γ2a production in each variant is accompanied by gene rearrangement (Fig. 2). The 5′ rearrangement breakpoints that we have mapped differ for each cell line, although all are within the region separating the MPC11 heavy chain variable region (V_{MPC11}) and constant region (Cγ2b) coding sequences. The 3′ rearrangement breakpoints also appear to be different for each class-switch variant, since the DNA restriction patterns on the 3′ side of the rearrangement sites

differ among the variants. From these data, we can conclude that the same phenotypic event, that is, a class switch, can take place in a single cell line through DNA rearrangement at any of several different sites.

An enhancer for heavy chain gene transcription has been mapped to the intervening sequences between the J_{H4} gene and the tandem repeat sequences 5′ of Cμ (3, 13, 25) (open boxes in Fig. 2). The class-switch rearrangement site in one of the MPC11 variants, 9.9.2.1, has led to deletion of this μ-derived enhancer element. The class switch in a second variant, 11.8, might also have resulted in deletion of the enhancer, since its rearrangement site falls within the region containing this enhancer. The three remaining MPC11 class-switch variants retain the μ-derived enhancer in their expressed heavy chain genes. MPC11 and all five of the variants produce comparable levels of immunoglobulin heavy chain (11; D. M. Zaller and L. A. Eckhardt, unpublished data).

Cloning and sequencing of the rearrangement site in a class-switch variant (9.9.2.1). As with all of the MPC11 class-switch variants, the *Hin*dIII fragment containing V_{MPC11} is retained unchanged in 9.9.2.1 (10; Fig. 2). However, the 2.1-kb *Xba*I fragment, which also contains these variable region coding sequences in MPC11, has been replaced in 9.9.2.1 by a much larger (6.9-kb) *Xba*I fragment (Fig. 2). Using these and additional mapping data, we placed the 9.9.2.1 rearrangement breakpoint within a 1-kb region bordered on the 5′ side by the retained *Hin*dIII recognition site and on the 3′ side by the missing *Xba*I site (10; Fig. 2).

We undertook further study of 9.9.2.1 because the 5′ rearrangement in 9.9.2.1 is several kb 5′ of any previously mapped class-switch site and because a description of its expressed gene might provide insight into its relationship to an intermediate cell line (9.7.1) that gives rise to γ2a-producing class-switch variants at high frequency (15). The 9.9.2.1 rearrangement site is contained within a 7.7-kb *Eco*RI fragment. Fragments of this size were isolated from a complete *Eco*RI digest of 9.9.2.1 genomic DNA and then cloned into a λ phage vector (Charon 16A). A restriction map of 9.9.2.1 DNA cloned in this way and which includes the V_{MPC11} gene and the 9.9.2.1 class-switch rearrangement site is shown in Fig. 3. This map and the map obtained by restriction analysis of total genomic DNA from 9.9.2.1 (Fig. 2) are in complete agreement. For example, the large *Xba*I fragment (6.9 kb) in 9.9.2.1 genomic DNA that hybridizes to pJ11 is present in cloned 9.9.2.1 DNA (Fig. 3). The *Hin*dIII fragment designated H3.2 in Fig. 3 contains the 9.9.2.1 class-switch site and, as expected, hybridizes with pJ11 (Fig. 4A).

As shown in Fig. 2, we knew from analysis of 9.9.2.1 genomic DNA that a maximum of 2.3 kb of the 7.7-kb *Eco*RI clone (recombinant phage clone Ch9.9.2.1) was derived from V_{MPC11} flanking sequences. Since 9.9.2.1 had switched from γ2b to γ2a expression, we expected that the remaining mouse DNA in Ch9.9.2.1 was derived from Cγ2a flanking sequences. Confirming this prediction, Sγ2a-3, an *Eco*RI fragment that maps immediately 5′ of Cγ2a, was found to hybridize to fragments H3.1 and H3.5 in Ch9.9.2.1 (Fig. 3 and 4A). Since Sγ2a-3 did not hybridize to H3.2, the *Hin*dIII fragment containing the 9.9.2.1 switch site (Fig. 4A), we concluded that the 3′ class-switch rearrangement breakpoint must lie 5′ of the Sγ2a-3 *Eco*RI fragment.

To determine the location of the 3′ breakpoint within germ line sequences, we subcloned the H3.2 fragment into a plasmid vector and used it as a hybridization probe against recombinant phage containing various segments of the mouse

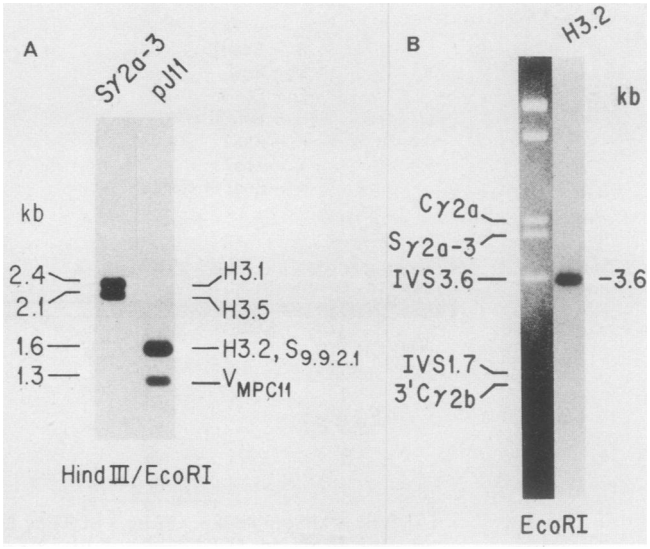


FIG. 4. (A) Hybridization of J_H region sequences and Cγ2a 5′ flanking sequences to Ch9.9.2.1. Ch9.9.2.1 DNA was digested with both *Hin*dIII and *Eco*RI, separated on agarose gels, and then transferred to a Gene Screen filter. As indicated above the gel tracks, the phage DNA was hybridized with either ^{32}P-Sγ2a-3 or ^{32}P-pJ11 (see Fig. 3). The 1.6-kb band that hybridizes with pJ11, H3.2, contains the 9.9.2.1 class-switch site. The 1.3-kb fragment that also hybridizes with this probe is the *Eco*RI-*Hin*dIII fragment that contains V_{MPC11}. (B) Localization of the $S_{9.9.2.1}$ 3′ breakpoint within germ line sequences. T. Honjo kindly provided us with a BALB/c germ line phage clone that contains Cγ2a and ca. 12 kb of its 5′ flanking sequences (ChMγ2a-9). DNA from this phage was cut with *Eco*RI, fractionated on agarose gels, and transferred to a Gene Screen filter. An ethidium bromide stain of the digest is shown (left lane), and the mouse *Eco*RI fragments within it are identified (e.g., the *Eco*RI fragment containing Cγ2a is designated "Cγ2a"; also, see Fig. 3). In the right lane is an autoradiograph of the Gene Screen filter after hybridization with ^{32}P-H3.2 (the 9.9.2.1 *Hin*dIII fragment that contains the class-switch site of this cell line).

germ line C_H region. The H3.2 fragment hybridized to an *Eco*RI fragment (IVS3.6) in the mouse germ line λ phage clone ChMγ2a-9, which contains Cγ2a and 5′ flanking sequences (35; Fig. 4B). The region of hybridization was further localized to the *Eco*RI-*Hin*dIII fragment forming the 5′ (as defined by the map in Fig. 3) half of this *Eco*RI fragment. Clearly, the 3′ breakpoint of the class-switch rearrangement in 9.9.2.1, like the 5′ breakpoint, is not within tandem repeat sequences. Also, note that the structure of the 9.9.2.1 gene shows no evidence of the γ2b to γ2a class switch through which it arose, since no γ2b flanking sequences are retained.

As shown in Fig. 3, the 3′ breakpoint involved in the 9.9.2.1 class switch lies 10.0 kb 5′ of Cγ2a sequences in germ line DNA, but only 6.5 kb 5′ of expressed Cγ2a sequences in 9.9.2.1. The difference is due to a deletion within H3.5. This deletion must have originated before isolation of the 9.9.2.1 cell line, since an identical lesion is present on the expressed chromosome in parental MPC11 DNA (Fig. 5). H3.5, itself 2.1 kb, hybridizes to a 2.1-kb *Hin*dIII fragment in MPC11 and 9.9.2.1 genomic DNA (Fig. 5) but to a 5.6-kb *Hin*dIII fragment in germ line DNA (data not shown). (The two additional bands detected with H3.5 in these experiments are derived from the "unexpressed" copy of chromosome 12 and are discussed below.) As shown in Fig. 3, the *Eco*RI fragment IVS1.7 is lost as a result of this deletion. Consequently, we were able to use IVS1.7 in other studies (7) as a

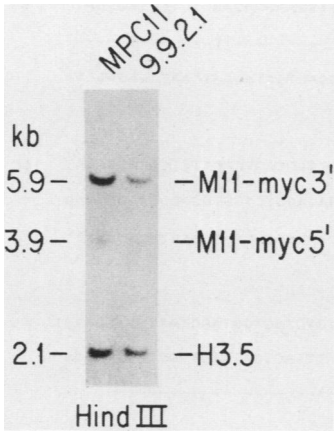


FIG. 5. Evidence of a deletion in Cγ2a 5′ flanking sequences that predates the MPC11 in vitro class switches. MPC11 and 9.9.2.1 genomic DNAs were cut with *Hin*dIII, fractionated on agarose gels, and transferred to nitrocellulose. ^{32}P-H3.2 was used as a hybridization probe. The 2.1-kb *Hin*dIII fragment detected with this probe is H3.5 itself. M11 *myc* 3′ and M11 *myc* 5′ refer to the reciprocal products of a translocation between chromosomes 12 and 15.

specific marker for the copy of chromosome 12 that was involved in a reciprocal translocation with c-*myc* on chromosome 15.

To more precisely define the sequences participating in the 9.9.2.1 class-switch rearrangement, we subcloned restriction fragments of H3.2 into M13 phage vectors and determined their sequences. The regions sequenced are diagrammed in Fig. 6. The germ line region into which the 5′ rearrangement breakpoint maps have previously been sequenced, and a compilation of these sequences, were obtained from GenBank. We compared our sequence of H3.2 with the GenBank sequences to determine the 9.9.2.1 5′ rearrangement breakpoint (Fig. 7). To locate the 3′ rearrangement breakpoint, we sequenced a 900-bp *Bam*HI-*Hin*dIII fragment isolated from the germ line *Eco*RI fragment IVS3.6 (regions sequenced shown in Fig. 6) and compared it with H3.2 DNA sequences (Fig. 7). Both breakpoints for the 9.9.2.1 class-switch rearrangement are indicated in Fig. 3, and DNA sequences surrounding them are shown in Fig. 7. The 9.9.2.1 switch site represents a precise joining of DNA at these two rearrangement breakpoints. Adjacent to the breakpoints is a 10-bp region of homology (for alignment, the two sequences must be shifted 1 bp relative to one another). At the site of the breakpoints, there is, in one case, the sequence AGC, and in the other case, the dinucleotide AA (Fig. 7). As discussed below (see Fig. 12), these short sequences are encountered again at the breakpoints of several additional MPC11 DNA rearrangements.

As was mentioned earlier, restriction enzyme maps of the heavy chain gene expressed in 9.9.2.1 (both by analysis of total genomic DNA and by analysis of cloned DNA) implied that this transcription unit no longer contained the transcriptional enhancer described by others (3, 13, 25). DNA sequence analysis confirmed this observation by showing that the loss of the *Xba*I recognition site mapping 5′ of this enhancer (Fig. 3) resulted not from a point mutation, but from deletion as a consequence of the 9.9.2.1 class-switch rearrangement.

Structure of the immunoglobulin heavy chain gene expressed in 9.7.1, an intermediate to the MPC11 class switch. 9.9.2.1 and 9.9.1.6.7 are both γ2a-producing cell lines that arose spontaneously from 9.7.1 (15). 9.7.1 is a mutagen (ICR191)-induced variant of MPC11 (29) that synthesizes an unusually large heavy chain; the 9.7.1 chain is 75,000 MW as compared with 55,000 MW for the heavy chain of MPC11 and other MPC11 class-switch variants. Earlier immunochemical studies of this heavy chain showed that it contained γ2a antigenic determinants (29). Our initial studies of genomic DNA from this variant (10) showed that the abnormal structure of the 9.7.1 heavy chain was the result of a gene rearrangement that fell within Cγ2b coding sequences. To determine the structure of this rearranged gene, we constructed a library of 9.7.1 genomic DNA cloned in Ch4 phage. A recombinant phage that hybridized to pJ11 was isolated and mapped by restriction enzyme analysis to yield the gene map shown in Fig. 3.

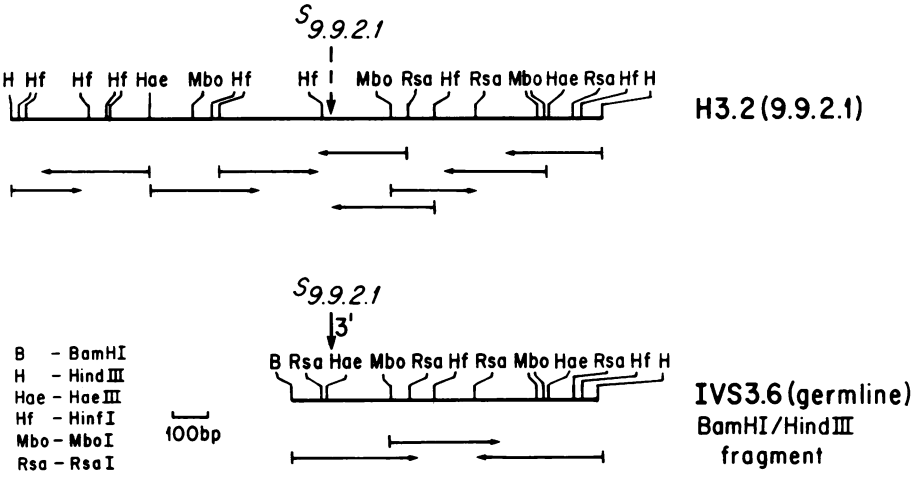


FIG. 6. Sequenced regions surrounding the class-switch site in 9.9.2.1. H3.2 is the *Hin*dIII fragment isolated from Ch 9.9.2.1 that contains the 9.9.2.1 class-switch site. The intact H3.2 fragment, or *Mbo*I, *Hae*III, *Rsa*I, or *Hin*fI digests of this fragment, were subcloned into M13 phage. Horizontal arrows indicate the direction and extent of the sequences obtained (by the dideoxy-chain termination method) from these clones. The *Bam*HI-*Hin*dIII fragment contained within IVS3.6 (see Fig. 3) was obtained from the mouse germ line phage clone ChMγ2a-9 and either cloned directly into M13 phage or cloned after *Mbo*I digestion. $S_{9.9.2.1}$ is the site of class-switch rearrangement in the 9.9.2.1 heavy chain gene. $S_{9.9.2.1}$ 3′ is the 3′ breakpoint of this rearrangement within germ line sequences.

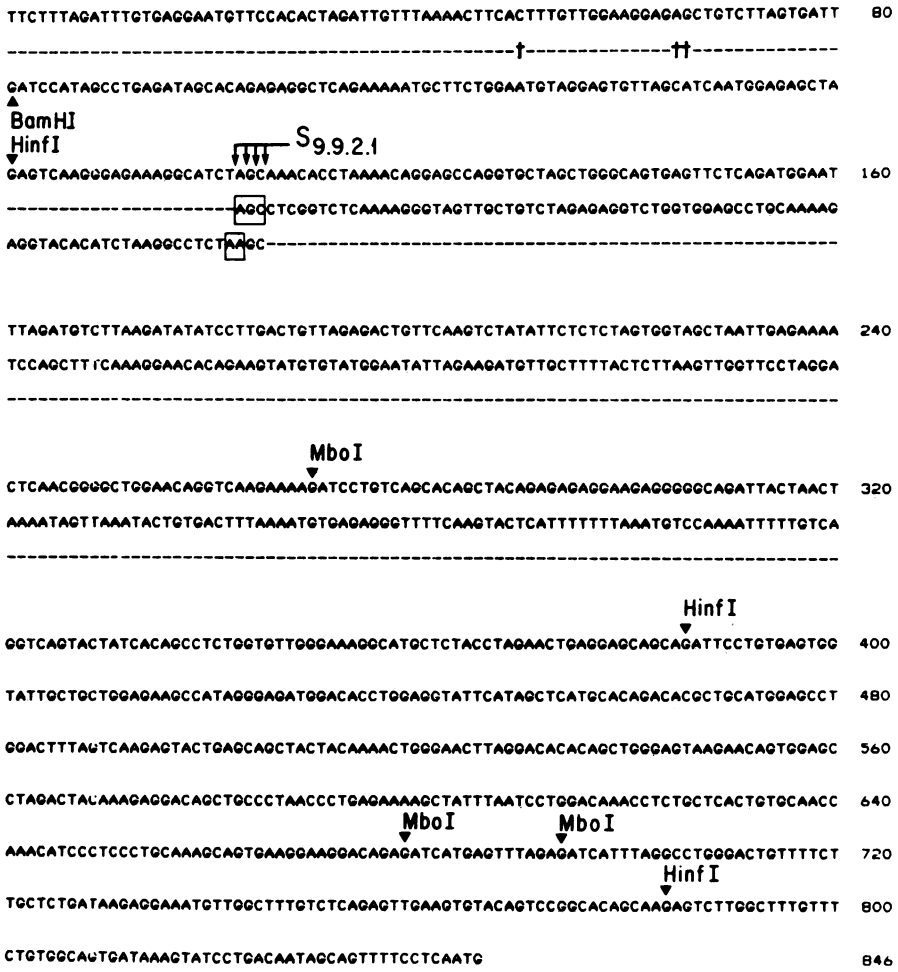


FIG. 7. Sequences surrounding the 5′ and 3′ breakpoints of the 9.9.2.1 class-switch rearrangement. A series of four arrows points to the 9.9.2.1 rearrangement site. Boxes enclose conserved sequences found at the breakpoints of several MPC11 rearrangements. Sequences are displayed 5′ to 3′ with respect to heavy chain gene transcription. Dashed lines indicate identity with the sequence presented on the top line (9.9.2.1). The 9.9.2.1 sequence (from H3.2) and the Cγ2a 5′ flanking sequence (from the *Bam*HI-*Hin*dIII fragment of IVS3.6) were determined as described in the text (see also Fig. 6). The J_H region sequence represents the compilation of several published and unpublished sequences and was obtained from GenBank. The Cγ2a 5′ flanking sequence shown begins at the *Bam*HI site of the *Bam*HI-*Hin*dIII fragment from IVS3.6. The other two sequences are aligned with it in a way that allows precise abutment of the $S_{9.9.2.1}$ 5′ and 3′ breakpoints. After nucleotide 320 in the 9.9.2.1 sequence, only the sequence of 9.9.2.1 is shown (nucleotides 321 to 846). Our sequence of 9.9.2.1 DNA (H3.2) and Cγ2a 5′ flanking DNA (IVS3.6) are identical throughout this region. †, Discrepancies between the 9.9.2.1 DNA sequence and the GenBank J_H region sequence.

From our genomic Southern data, we knew that the 9.7.1 rearrangement had occurred 3′ of the *Bgl*I site within the hinge exon of Cγ2b. This *Bgl*I site maps within a 3.8-kb *Eco*RI fragment (R1-S9.7.1) in the Ch9.7.1 clone (Fig. 3). Since the 9.7.1 heavy chain contains γ2a determinants, we again used Sγ2a-3, the *Eco*RI fragment 5′ of Cγ2a, to ask whether the 9.7.1 rearrangement involved sequences flanking Cγ2a. As shown (Fig. 8), this probe hybridized to the 3.8-kb *Eco*RI fragment containing the 9.7.1 switch site, suggesting that the 3′ rearrangement breakpoint in this variant, unlike that in 9.9.2.1, fell within Sγ2a-3. We further mapped the rearrangement site in this *Eco*RI fragment (RI-S9.7.1), both by comparative restriction enzyme mapping of it and Sγ2a-3 and by DNA sequencing. The regions sequenced are shown in Fig. 9, and the sequences are shown in Fig. 10. The 5′ rearrangement breakpoint falls within the Cγ2b gene, for which published sequences are available (GenBank). As shown in Fig. 3, this breakpoint maps within the Cγ2b hinge exon, eliminating by rearrangement some of the coding sequences within this exon, as well as the 3′ donor splice site of the exon.

The 3′ rearrangement breakpoint in the expressed heavy chain gene of 9.7.1 falls within a region flanking Cγ2a that has not been sequenced previously. We subcloned and sequenced this region from the ChMγ2a-9 phage (Fig. 9 and 10). From these data, we determined that the 3′ breakpoint lies 3 kb 5′ of Cγ2a, nearly 1 kb downstream from the tandem repeat sequences flanking Cγ2a. As in the 9.9.2.1 rearrangement, the sequences AGC and AA, respectively, are found at the3′ and 5′ breakpoints.

The 9.7.1 rearrangement results in a new transcription unit that contains the V_{MPC11} exon, the first (C_H1) exon and a part of the hinge (H) exon of Cγ2b, and all of Cγ2a coding sequences. Although we have not determined the precise structure of the heavy chain mRNA produced in this cell line, its large size (~2.2 kb, as compared with 1.8 kb for MPC11 γ2b mRNA) (data not shown), as well as the large size of its translational product (the 9.7.1 heavy chain,

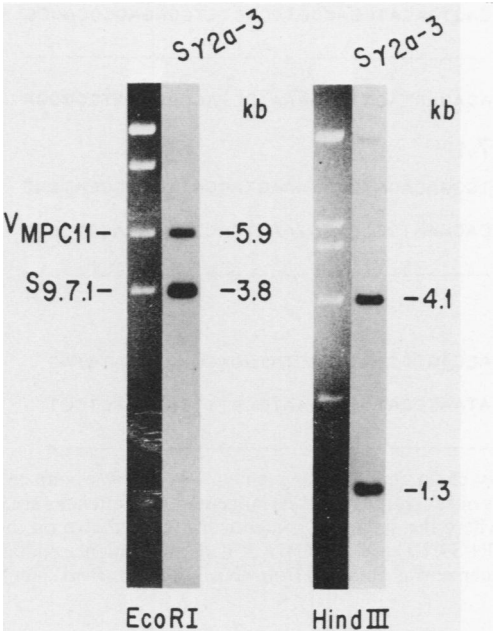


FIG. 8. Hybridization of Cγ2a 5′ flanking sequences to Ch9.7.1. Ch9.7.1 DNA, cut with either *Eco*RI or *Hin*dIII, was run on an agarose gel and transferred to a Gene Screen filter. The restriction digests, stained with ethidium bromide, are shown adjacent to autoradiographs of the same DNA after transfer and hybridization with ^{32}P-Sγ2a-3. The tandem repeat sequences in Sγ2a-3 bear some homology with those lying 5′ of Cγ2b. As a result, the 5.9-kb *Eco*RI fragment that contains both the latter sequences and V_{MPC11} hybridizes to Sγ2a-3 (see Fig. 3). The same cross-homology is responsible for the 1.3-kb band seen in Sγ2a-3 hybridizations to *Hin*dIII-digested Ch9.7.1 DNA. The 3.8-kb (*Eco*RI) and 4.1-kb (*Hin*dIII) fragments in Ch9.7.1 contain the 9.7.1 rearrangement site.

75,000 MW), suggest that either C_H1 alone or both C_H1 and H Cγ2b exons are retained in 9.7.1 heavy chain mRNA. Since the C_H1 exon is undisturbed, there is no a priori reason to suppose that this exon is excluded from the mRNA and heavy chain polypeptide. The splice junction at the 5′ side of the hinge exon is also unchanged, but if it is used to splice this exon to the γ2b C_H1 exon, a new splice site must be used on the 3′ side of the abbreviated hinge to avoid early termination during translation. The ninth codon after the rearrangement site in 9.7.1 is a termination codon.

DNA sequence of c-*myc*/C_H region chromosome translocation breakpoints. Cγ2a flanking sequences are not only involved in DNA rearrangements associated with class switching in MPC11; they have also been the subject of other types of rearrangements in this cell line. Earlier in this report, we described a deletion (Fig. 3) of ~3.5 kb which occurred ~5.0 kb 5′ of Cγ2a on the expressed C_H chromosome of MPC11. The comparable region on the unexpressed C_H chromosome has undergone a reciprocal translocation event with chromosome 15, within sequences coding for the oncogene c-*myc* (37). In the experiment shown in Fig. 5, the reciprocal products of this translocation are represented by two of the three *Hin*dIII fragments detected in MPC11 DNA with the H3.5 hybridization probe.

Physical evidence for this translocation event, as well as its localization to the unexpressed copy (with respect to immunoglobulin expression) of chromosome 12, came from analysis of a phage clone (M11 *myc* 5′) that we isolated from the 9.7.1 genomic library (37). This clone contains a Cγ2b gene and a portion of the first exon of c-*myc*. DNA sequence analysis of this and the reciprocal product (M11 *myc* 3′) of this chromosome exchange has been reported (37). At the point of recombination, 11 bp of the c-*myc* sequence have been deleted, as well as ca. 300 bp of the chromosome 12-derived sequences. Although the latter sequences are clearly part of the tandem repeat region flanking Cγ2a, a precise localization was not possible given the available DNA sequences of this region. As a result, we sequenced a portion of this region and localized the M11 *myc* 3′ breakpoint within it (Fig. 11). The nucleotides AGC and AA found at the rearrangement breakpoints of class-switch variants 9.7.1 and 9.9.2.1 were once again found at the breakpoints of this oncogene-immunoglobulin locus rearrangement (Fig. 12). Our sequencing of Cγ2a flanking sequences has not yet led to identification of the comparable M11 *myc* 5′ breakpoint.

DISCUSSION

We examined the expressed immunoglobulin heavy chain genes of several γ2a-producing variants, all derived from a single γ2b-producing parental cell line (MPC11). In each of these variants, the switch from γ2b to γ2a production is accompanied by gene rearrangement. The 5′ breakpoints of these class-switch rearrangements are scattered over a ca. 2.4-kb region, and most lie outside of tandem repeat sequences flanking Cγ2b. Only one of the variants (9.91.6.7) has undergone rearrangement within these repeat sequences.

In the class-switch variant 9.9.2.1, the 3′ rearrangement breakpoint maps ~10 kb 5′ of Cγ2a. This places it consid-

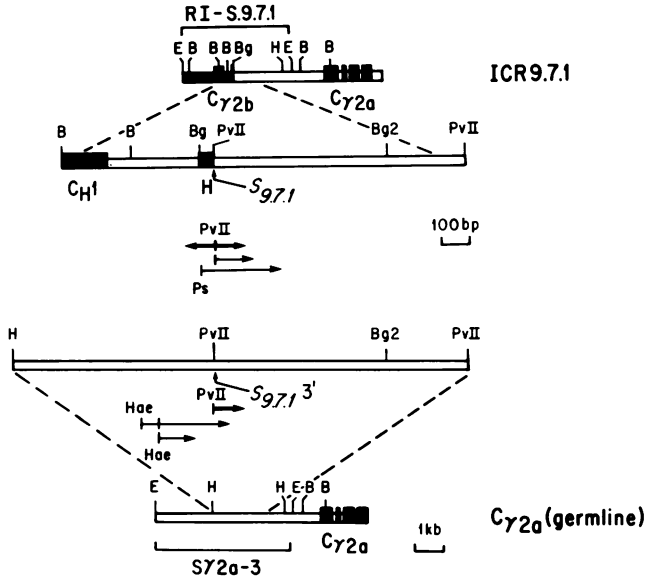


FIG. 9. Sequenced regions surrounding the 9.7.1 rearrangement site. RI-S9.7.1 is the *Eco*RI fragment in Ch9.7.1 that contains the 9.7.1 rearrangement site. It was subcloned into pBR325 and compared by both restriction enzyme analysis and DNA sequencing to Sγ2a-3. Thick horizontal arrows indicate the extent and direction of sequences obtained by the method of Maxam and Gilbert (21). Thin horizontal arrows similarly indicate the extent and direction of sequences obtained by dideoxy-chain termination sequencing of *Pvu*II (9.7.1), *Pst*I (9.7.1), and *Hae*III (Sγ2a-3) fragments subcloned in M13 phage. $S_{9.7.1}$ marks the site of rearrangement in the 9.7.1 heavy chain gene and $S_{9.7.1}$ 3′ marks the 3′ breakpoint of this rearrangement within germ line (Sγ2a-3) sequences.

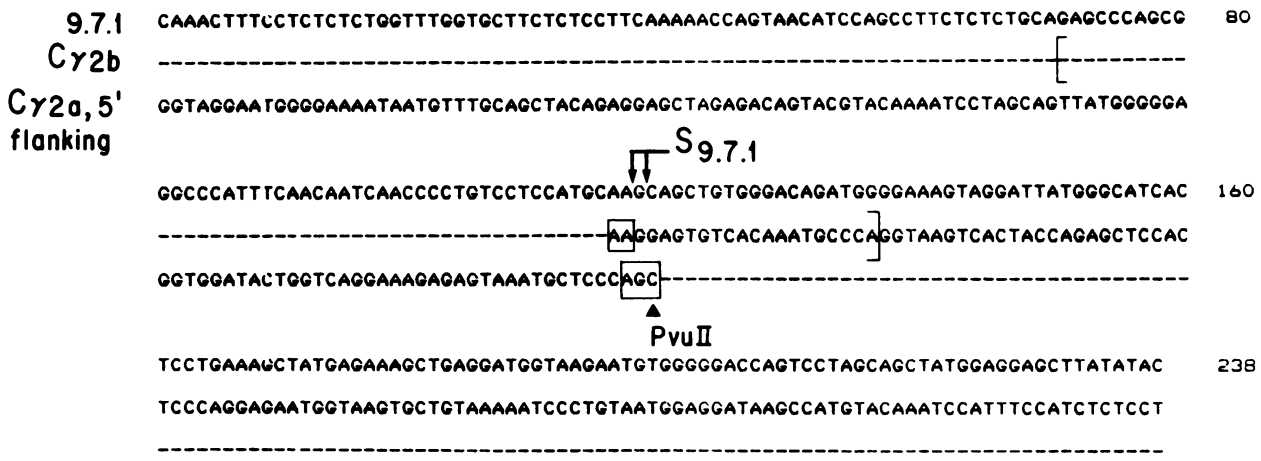


FIG. 10. Sequences surrounding the 5′ and 3′ breakpoints of the 9.7.1 heavy chain gene rearrangement. Two arrows point to the 9.7.1 rearrangement site. Boxes enclose conserved sequences found at the breakpoints of several MPC11 rearrangements. Sequences are displayed 5′ to 3′ with respect to heavy chain gene transcription. Dashed lines indicate where the sequence is identical to that shown on the top line (9.7.1). The Cγ2b hinge exon is bracketed. The 9.7.1 sequence (obtained from RI-S9.7.1) and the Cγ2a 5′ flanking sequence (obtained from Sγ2a-3) were determined as described in the text (see also Fig. 9). The Cγ2b sequence was obtained from GenBank. The *Pvu*II site that maps near the $S_{9.7.1}$ 3′ breakpoint (Fig. 9) is indicated.

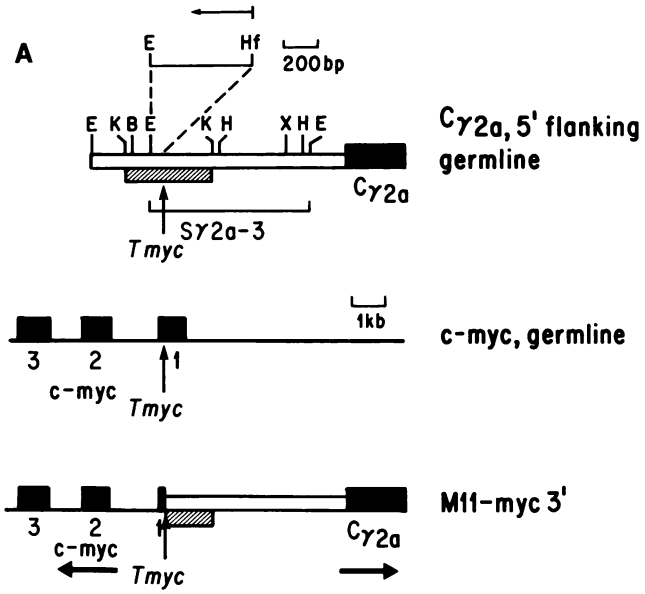
erably 5′ of the tandem repeat sequences flanking Cγ2a. We have tentatively placed the 3′ rearrangement breakpoint of 11.8 (Fig. 2) between 9 and 9.5 kb upstream of Cγ2a, since the restriction map of the expressed heavy chain gene in 11.8 corresponds to that of the region flanking the MPC11 Cγ2a gene shown in Fig. 3 (also data not shown). A single difference in the two maps is a *Hin*dIII site which borders the 0.5-kb region containing the 11.8 switch site. However, recent studies have shown that, on the expressed chromosome of MPC11, the Cγ2a gene has been tandemly duplicated (S. A. Tilley and B. K. Birshtein, unpublished data). The new restriction pattern that results from this duplication accounts for this *Hin*dIII site and implies that 11.8 has used a second copy of the Cγ2a gene for switching. Again, the 11.8 3′ breakpoint is several kb removed from tandem repeat sequences.

The class-switch rearrangements in 9.9.2.1 and 9.7.1 and likely 11.8 involve the abutment of sequences within the MPC11 expressed heavy chain gene and Cγ2a flanking sequences. M224, M319.2, and 9.9.1.6.7 may have undergone more complex rearrangements, since their restriction maps 3′ of the rearrangement sites (Fig. 2) do not coincide with that of the region flanking Cγ2a in MPC11. However, we have evidence to suggest that in M224 and M319.2, the 3′ rearrangement breakpoints lie outside of tandem repeat sequences, since neither shows rearrangement within a *Bam*HI fragment containing these sequences.

Much attention has been given to tandem repeat sequences in this discussion because of studies mapping the class-switch sites in several independently isolated mouse myelomas. The breakpoints for these rearrangements generally fall within or near regions that consist of short DNA sequences repeated in a tandem array (tandem repeat sequences) (reviewed in references 20 and 26). Stretches of tandemly arranged repeats, constituted of 10- to 80-bp units, are found 5′ of each of the C_H genes (with the exception of Cδ) (27). Although the length and sequence structure of the repeat unit differ for each gene, there are short sequence elements shared among them. It has been proposed that homologous pairing between these shared elements promotes the associations between C_H genes that result in a class switch. They have, therefore, been viewed by some as at least one of the structural requirements for the heavy chain class switch. However, as we showed in an earlier report (10) and provide additional evidence for here, tandem repeat sequences are, for the most part, not the site of DNA rearrangement in MPC11 class switches occurring in culture.

This has been seen as well in other cultured cell class-switching systems. In one study (32), genomic DNA from a spontaneously arising hybridoma class switch variant (γ3 to γ1) was analyzed. The Cγ3 gene expressed in the parental cell line was deleted in the γ1-producing variant. However, the tandem repeat regions flanking Cγ1 remained unaffected, suggesting that the class switch in this cell line lies outside of tandem repeat sequences. Spontaneous class switching has also been described (1, 2, 6) in Abelson virus-transformed cell lines, which are generally regarded as representative of pre-B lymphocytes. Restriction analysis of genomic DNA in such cell lines that have undergone a class switch suggests that some type of class-switch DNA rearrangement has taken place (1, 6). Again, however, no evidence of rearrangement within the tandem repeat sequences is seen.

Taken together, the available data from studies of cultured cell class switches imply that many sites within heavy chain genes (including coding regions, e.g., 9.7.1) can meet the primary structural requirements for class switching. What appears to be the use of only a portion of these sites (those in tandem repeats) in many myelomas may be a reflection not of the necessity for these sites to lie within repeat sequences, but rather of the fact that the repeat regions themselves comprise a major portion of the DNA flanking the C_H genes. For example, in germ line DNA, tandem repeats occupy ca. 45% of the intron separating J_H genes and Cμ (26). Accordingly, cells that undergo class-switch rearrangement at sites outside of tandem repeats often arise from precursors in which the relevant repeat regions are much reduced with respect to germ line. In MPC11, the tandem repeat region in the 6.1-kb intron separating V_{MPC11} and Cγ2b is ca. 1.0 kb, having been truncated by a previous class-switch rearrangement (the μ to γ2b switch in an MPC11 precursor). The repeat region flanking Cγ2a has been reduced in size by an internal deletion. In MPC11, then, because of these deletions, it becomes possible to identify sites outside of tandem repeats that, like those



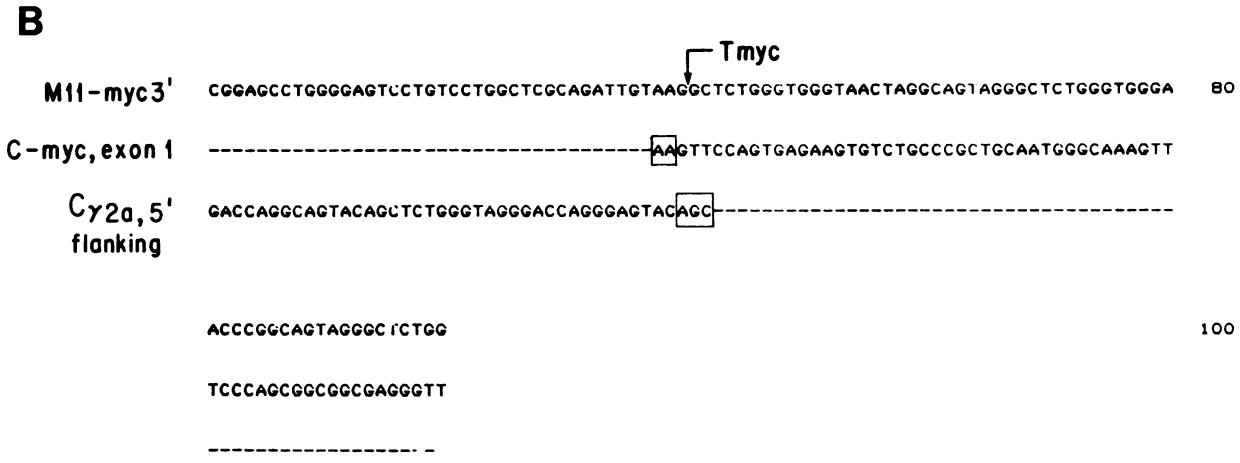


FIG. 11. Sequences surrounding the breakpoints of a c-*myc*-C_H chromosome translocation in MPC11. One of the reciprocal products of the c-*myc*-C_H chromosome translocation in MPC11 consists of the 5′ end of c-*myc* exon 1 (with 5′ defined by c-*myc* transcription) recombined with Cγ2a flanking sequences (M11 *myc* 5′). The other product contains the 3′ end of c-*myc* exon 1, as well as c-*myc* exons 2 and 3, combined with Cγ2a flanking sequences (M11 *myc* 3′). The DNA sequences surrounding the breakpoints of the latter translocation product are shown. Sequences are displayed 5′ to 3′ relative to heavy chain gene transcription (opposite to c-*myc* transcription). Dashed lines indicate where the sequence is identical to that shown on the top line (M11 *myc* 3′). The M11 *myc* 3′ and c-*myc* exon 1 sequences were published previously (37). The Cγ2a 5′ flanking sequence was obtained by dideoxy-chain termination sequencing of a *Hin*fI-*Eco*RI fragment which had been subcloned from Sγ2a-3 into an M13 phage vector. Boxes enclose conserved sequences found at the breakpoints of several MPC11 rearrangements. (These sequences are also present at both breakpoints when the immunoglobulin and *myc* sequences are displayed 5′ to 3′ with respect to c-*myc* transcription.)

within tandem repeats, provide the structural elements required for a class-switch rearrangement.

A reduction in the size of tandem repeat regions such as that seen in MPC11 may well be a common feature of B-lymphocyte differentiation. Hurwitz and Cebra (14) report that surface IgM-positive spleen cells, when stimulated with lipopolysaccharide (a polyclonal B-cell mitogen), give rise to a significant population of plasmablasts that continue to synthesize IgM but have undergone deletion in the J_H-Cμ intron, 45% of which, as noted above, consists of tandem repeats. Since lipopolysaccharide-stimulated cultures also give rise to cells that have switched from μ to another class of heavy chain, one interpretation of these data is that deletions in tandem repeats precede class-switch rearrangements. In support of this interpretation are reports of μ-producing Abelson virus-transformed cell lines which have large deletions in the J_H-Cμ intron (as much as 60% of the total length) and which then switch to γ2b heavy chain production (6). This could be viewed as a cause-effect relationship—deletions in tandem repeat sequences leading to class switching—or the preliminary deletions in tandem repeat sequences might simply serve as an indicator of enzyme conditions in the cell, signaling that the enzymes required for class switching are now activated and operating. As mentioned earlier, at least some of the class-switch rearrangements in the γ2b-producing Abelson lines seem to

have breakpoints outside of tandem repeats (1, 6). Whether and where most LPS-stimulated cells undergo class-switch DNA rearrangements are not known.

Clearly, there are some constraints on where class-switch recombination can occur if efficient immunoglobulin gene expression is to result. Recently, an enhancer for immunoglobulin heavy chain gene transcription has been identified 5′ of the tandem repeat sequences flanking Cμ (3, 13, 25). Thus, bordering the repeats on either side are at least two types of sequences whose interruption might lead to loss of immunoglobulin gene expression: the enhancer sequences on the 5′ side and the constant region coding sequences on the 3′ side. Class-switch recombination that occurs within either enhancer or Cμ sequences will likely have a deleterious effect on immunoglobulin gene expression (e.g., reference 2). Conversely, rearrangements with breakpoints within the tandem repeats would presumably have no such effect. Again, the use of sites within tandem repeats would not reflect a primary structural requirement for these sequences in the class switch event. However, selection by the immune system for cells that continue to produce antibody would tend to favor cells that had undergone rearrangement in these presumably nonfunctional regions. The alternate outcome to class-switch type rearrangements expected then is loss of immunoglobulin expression, and this frequently occurs in myeloma cells. Nonimmunoglobulin-producing B lymphocytes may be the normal genetic "price" paid for using gene rearrangement as a means for creating antibodies of many classes and antigen specificities.

Implicit in this discussion of the similarities and disparities between our general understanding of the class switch as it occurs in animals and the more directly analyzed class switch that occurs in culture is the possibility that the two are the result of two inherently different mechanisms. As mentioned above, cells that have switched heavy chain class are not the only kind of phenotypic variants seen arising spontaneously in cultures of myeloma cells. Spontaneous variants of MPC11 that no longer secrete heavy chains arise at a rate of 10^{-3} per cell per generation (4). Preliminary analysis of such variants indicates that a significant proportion of these cells have undergone some type of gene rearrangement (H. Yu and L. A. Eckhardt, unpublished data). As the instability of these genes is not shared by other genes expressed in these cells (4), we think that it is likely that this instability reflects the action of the same (or perhaps partially modified) DNA rearrangement "machinery" which is similarly specific for the immunoglobulin genes in normal B cells. In this regard, it would be informative to determine whether spontaneous mutations resulting from the same types of rearrangements occur in genes expressed by other differentiated cell types.

Two of the MPC11 class-switch variants, 9.9.2.1 and 11.8, have undergone rearrangement in or near the μ-derived enhancer for heavy chain gene transcription. Surprisingly, both synthesize heavy chains in quantities comparable to that produced by the MPC11 parent. We cloned the expressed heavy chain gene from one of these cell lines (9.9.2.1) and showed that this transcriptional enhancer is lost as a result of the class-switch rearrangement. If efficient expression of heavy chain genes is dependent on an enhancer element, then it follows that the same rearrangement may have led to replacement in 9.9.2.1 of the μ-derived enhancer with a second, similar element. Alternatively, the μ-derived enhancer, defined in transfection experiments, may be unnecessary for immunoglobulin heavy chain gene expression in its normal chromosomal milieu. The existence

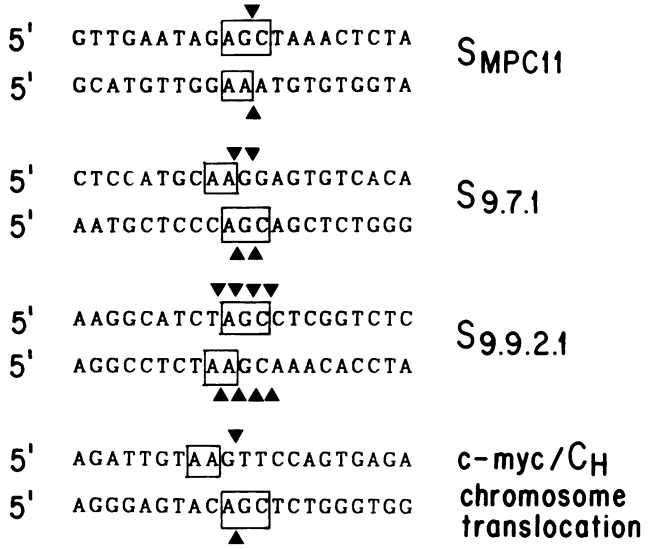


FIG. 12. Conserved sequences at the breakpoints of several MPC11 DNA rearrangements. All sequences are displayed 5′ to 3′ with respect to immunoglobulin heavy chain gene transcription. Arrowheads mark the rearrangement breakpoints. Sequences surrounding the 5′ breakpoint are shown on the top line of each pair of sequences, and those surrounding the 3′ breakpoint are shown on the lower line. The class-switch rearrangement breakpoints in 9.9.2.1 and 9.7.1 were determined in the present study, as was one of the breakpoints of the MPC11 c-*myc*-IgH chromosome translocation. The site of the class-switch rearrangement responsible for the establishment of MPC11 as a γ2b-producing cell (S_{MPC11}) was sequenced previously by others (16).

of a heavy chain transcriptional enhancer within the 9.9.2.1 J_H-Cγ2a intron is presently being investigated. The identification of additional enhancer regions within the C_H locus may also help explain why chromosome translocation between this locus and the c-*myc* oncogene locus on chromosome 15 often results in increased c-*myc* expression. The enhancer sequences already identified in the region between J_H and Cμ are not implicated in most translocations of this type, since they are not found adjacent to the expressed portion of the translocated c-*myc* gene.

The MPC11 class-switch variants 9.9.2.1 and 9.9.1.6.7 arose through an intermediate cell line (9.7.1) that produces an abnormally large heavy chain. We have been interested in the expressed heavy chain gene of 9.7.1 both because of the unusual size of the 9.7.1 heavy chain and because of the very high spontaneous frequency at which cells producing normal γ2a heavy chains arise among ICR9.7.1 progeny (1 of 900: 15). This frequency is ca. 100 times that of similar cells arising in MPC11 (parental) cultures. We isolated the gene coding for the aberrant 9.7.1 heavy chain and showed that it is the product of an unusual class-switch rearrangement in which the 5′ rearrangement breakpoint falls within coding sequences (the hinge exon of Cγ2b). The 3′ rearrangement breakpoint is ~3 kb 5′ of the Cγ2a gene. Although 9.9.2.1 is a spontaneous variant of 9.7.1, it retains sequences 5′ of Cγ2a that are absent from the 9.7.1 heavy chain gene. Since we have evidence that tandem copies of Cγ2a genes are present on the expressed chromosome of MPC11 (Tilley and Birshtein, unpublished results), this difference in gene structure most likely results from use of different Cγ2a genes in 9.7.1 and 9.9.2.1.

The heavy chain transcription unit of 9.7.1, then, includes V_{MPC11}, partial γ2b constant region sequences, and an intact

Cγ2a gene. We have not formally proven that all of these coding sequences are included in the 9.7.1 heavy chain protein or mRNA, or both. However, both molecules are larger than the comparable protein and mRNA of MPC11, suggesting that at least some of the Cγ2b sequences that remain are both transcribed and translated in 9.7.1. An explanation for 9.7.1 serving as an efficient intermediate in class switching is suggested by this gene structure. In MPC11, class switching from γ2b to γ2a requires gross gene rearrangements. In 9.7.1, the structure of the transcription unit is such that not only additional rearrangements, as seen in 9.9.2.1, could lead to synthesis of a γ2a heavy chain, but also minor changes around the splice junctions of the Cγ2b-derived exons could result in γ2a heavy chain production. Loss of protein domains resulting from a change in RNA splicing patterns has been seen in several systems, including the immunoglobulin and globin gene families.

We have found evidence for limited sequence conservation at the site of DNA breakage and rejoining in four of five rearrangements sequenced in MPC11. In these rearrangements, a triplet (AGC) is found at the breakpoint on one of the participating DNA molecules, and the dinucleotide AA is found on the other (Fig. 12). This is true for both the class-switch rearrangement that has taken place in a γ2a-producing cell line (9.9.2.1) and for the DNA rearrangement found in the class-switch intermediate through which this γ2a-producing cell arose (9.7.1). Neither of these rearrangements involved tandem repeat sequences. Notably, AGC and AA are also found at the breakpoints of two gene rearrangements (both involving tandem repeat sequences) that presumably took place in the MPC11 line before its establishment as an IgG2b-producing tumor. One of these is the μ to γ2b class switch that established this as a γ2b-producing cell, and the other is a reciprocal chromosome translocation involving the oncogene c-*myc* and the immunoglobulin C_H locus. The class-switch rearrangement breakpoints in some, but not all, mouse myelomas studied so far are also bordered by these sequences (reviewed in references 12 and 27). (The unstable nature [19] of the tandem repeat regions within which many of these breakpoints map makes it difficult to determine whether these are the primary sites of class-switch rearrangement or the sites of secondary deletions following that event.) One MPC11 DNA rearrangement that appears not to employ these sequences is a rearrangement involving the tandem repeat sequences flanking Cγ3 and Cγ2b on the unexpressed copy (with respect to immunoglobulin genes) of chromosome 12 (16). The mechanistic relationship between the immunoglobulin heavy chain class switch and c-*myc* rearrangements in immunoglobulin-producing tumor cells has recently been the subject of much speculation. Consequently, it is of interest that the c-*myc* rearrangement (chromosome translocation) in MPC11 involves a sequence that is shared by both in vitro (in culture) and in vivo class-switch gene rearrangements in the same cell line.

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