Molecular Basis of Heavy-Chain Class Switching and Switch Region Deletion in an Abelson Virus-Transformed Cell Line

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We demonstrated that a subclone of an Abelson murine leukemia virus-transformed B-lymphoid cell line switched from μ to γ_{2b} expression in vitro, by the classical recombination-deletion mechanism. In this line, the expressed V_HDJ_H region and the $C_{\gamma2b}$ constant region gene were juxtaposed by a recombination event which linked the highly repetitive portions of the S_μ and $S_{\gamma2b}$ regions and resulted in the loss of the C_μ gene from the intervening region. An additional recombination event in this subclone involved an internal deletion in the S_μ region of the expressed (switched) allele. One end of this deletion occurred very close to the switch recombination point. Despite the recombination-deletion mechanism of switching, the γ_{2b}-producing line retained two copies of the C_μ gene and two copies of the sequence just 5' to the $S_{\gamma2b}$ recombination point. The possible significance of the retention of these sequences to the mechanism of class switching is discussed.

During the differentiation of a B-lymphocyte to a plasma cell, the class of immunoglobulin produced by its progeny may change from immunoglobulin M (IgM) and IgD to a different class (e.g., an IgG or IgA), while maintaining the same variable region specificity (13, 32). This phenomenon, termed heavy-chain class switching, allows a single clone of B-cells to generate progeny which maintain the same antigen-binding specificity (V_H) linked to different C_H effector functions.

The order of the C_H genes has been determined to be $5'-(V_HDJ_H)-C_\mu-C_\delta-C_{\gamma3}-C_{\gamma1}-C_{\gamma2b}-C_{\gamma2a}-C_\epsilon-C_\alpha-3'$ (38). Much of our current knowledge of the molecular basis of heavy-chain class switching has come from comparative studies of heavy-chain gene structure in independently derived immunoglobulin-secreting myeloma tumors (25, 37). These studies have indicated that the switch process is often effected by a recombination-deletion mechanism distinct from that involved in V_HDJ_H joining (2, 3, 25, 37). Expression of a given downstream C_H gene in a myeloma results from juxtaposition of the C_H gene to the expressed V_HDJ_H complex by deletion of the intervening DNA sequence, including the C_μ, and any other intervening C_H genes (12, 14, 23, 42). This process has been proposed to be mediated by a set of repeated sequences (S regions) which lie several kilobases (kb) 5' to each C_H gene (24, 28). In its most simple form, switching could occur by direct joining of the switch recombination sequence 5' to C_μ to those 5' to the expressed C_H gene, resulting in a linear deletion of the intervening DNA (subsequently referred to as a classical recombination-deletion mechanism). However, the occurrence of switch recombination sequences from both upstream and downstream C_H genes 5' to an expressed γ_1 gene of a myeloma indicates that the process, at least occasionally, may be more complicated (15, 31). Various models for class switching have been proposed to accommodate the complicated structures, including unequal exchanges between sister chromatids or chromosomes (15, 31).

The 18-81A2 line is a γ_{2b}-producing derivative of the μ-producing 18-8 Abelson murine leukemia virus (A-MuLV)-transformed cell line. We have previously shown that this subclone produces γ_{2b} heavy chains containing a variable region sequence which derives from the same V_HDJ_H-rearranged heavy-chain allele that is used for μ heavy-chain production by the parental line (5). The A2 line also has a V_HDJ_H rearrangement at its other heavy-chain allele, but this rearrangement is nonfunctional due to an in-phase termination codon in the D segment (6). Because the A-2 line apparently contained two copies of the C_μ gene, we suggested that the switch to γ_{2b} production in this line might have occurred, at least in part, by a differential RNA-processing mechanism (5) similar to that used for the simultaneous expression of μ and δ by B-lymphocytes (20, 23, 27). In this report, we demonstrate that although the A2 line clearly retains two copies of the C_μ gene as well as two copies of $S_{\gamma2b}$ sequences, the class switch in this line occurs by the classical recombination-deletion mechanism.

MATERIALS AND METHODS

Cells. Cell lines were grown as a suspension in RPMI medium supplemented with 10% heat-inactivated fetal calf serum and 0.0004% (vol/vol) 2-mercaptoethanol. Subcloning of cell lines was performed by plating at limiting dilution in microtiter wells. Further details about the growth and characteristics of the lines have been presented elsewhere (5, 7).

Analysis of heavy-chain gene organization. DNA preparation, restriction enzyme digestions, agarose gel electrophoresis, DNA blotting procedures, probe preparations, and hybridization procedures were performed as described previously (7, 22). The C_μ-probe was prepared from the 1,250-base-pair (bp) insert of pABμ-8 (4). The J_H probe was prepared from the 3'-terminal 700 bp of the 6.2-kb EcoRI containing the germ line J_H segments (8), and the 5'- and 3'-$S_{\gamma2b}$ probes were prepared from the 0.8- and 1.2-kb EcoRI-BglII fragments, respectively, derived from the 4.1-kb EcoRI $S_{\gamma2b}$ portion of the genomic clone, SL1 (39) (see Fig. 2A). Further details are described in the figure legends.
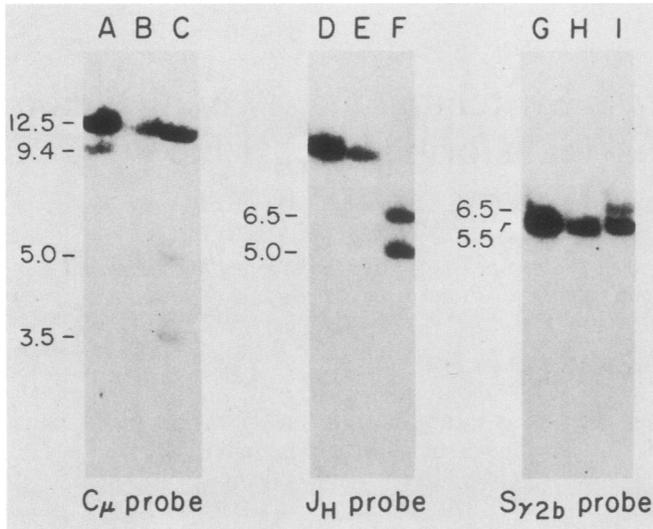
* Corresponding author.

FIG. 1. Heavy-chain gene organization in A-MuLV transformants. Approximately 10 μg of genomic DNA from mouse liver (lanes A, D and G), 18-8 (lanes B, E and H), and A-2 (lanes C, F and I) was digested with BamHI, fractionated by electrophoresis through 1.0% agarose gels, and blotted to nitrocellulose paper; duplicate filters were assayed respectively for hybridization to the ^{32}P-labeled inserts from pABμ-1(C_μ probe, lanes A through C), PJ$_H$800 (J$_H$ probe, lanes D through F), or 3'S$_{\gamma2b}$ probe (lanes G through I).

Genomic cloning and DNA sequencing. Genomic libraries were constructed by cloning an MboI partial digest of genomic DNA from the 18-81 A2 line into the lambda phage vector Charon 30 (34) as described by Blattner et al. (10). Recombinant phage were screened by the method of Benton and Davis (9) for hybridization to J$_H$ and 3'-S$_{\gamma2b}$ probes. Plaques hybridizing to both probes were purified, and appro-

priate portions of the inserts were subcloned into pBR322 and mapped by digestion with various restriction enzymes as described previously (6). Various regions of the inserts were sequenced by the method of Maxam and Gilbert (26) as previously described (6). For further details, see the legends to Fig. 2 and 3.

RESULTS

The class switch in the A2 line is accompanied by the movement of the S$_{\gamma2b}$ region into close proximity with the expressed V$_H$ region. The nature of the DNA alterations that accompanied the class switch was studied in the following manner. Genomic DNAs from liver, 18-8, and A-2 were digested with various restriction endonucleases, fractionated by agarose gel electrophoresis, and transferred to nitrocellulose paper, and duplicate filters were assayed for hybridization to ^{32}P-labeled C_μ, J$_H$, and 3'-S$_{\gamma2b}$ probes (Fig. 1). BamHI cuts between J$_H$2 and J$_H$3 and in the middle of the second C_μ exon but not in the intervening region (Fig. 2) (6). Thus, BamHI-digested liver DNA displayed a C_μ-positive fragment of 12.5 kb, which corresponds to the sequences 3' to the BamHI site in C_μ, as well as a 9.4-kb fragment that contained the 5' portion of the C_μ-coding region and the 3' portion of the J$_H$ region (Fig. 1, compare lanes A and D). When assayed with the J$_H$ and C_μ probes, DNA from the parental 18-8 line was found to have a hybridization pattern that was essentially identical to that of liver DNA, i.e., no obviously rearranged bands (Fig. 1, lanes B and E; the 9.4-kb band in lane B was more evident in longer exposures). This result can be explained by the fact that the V$_H$ segment of the productive V$_H$DJ$_H$3-rearranged allele of 18-8 (A2) has a BamHI site ca. 250 bp from the joint, whereas the nonproductive allele contains a V$_H$DJ$_H$2 rearrangement (6) (Fig. 2A). Thus, the nonproductive allele will yield an embryonic fragment which hybridizes to the J$_H$ and C_μ
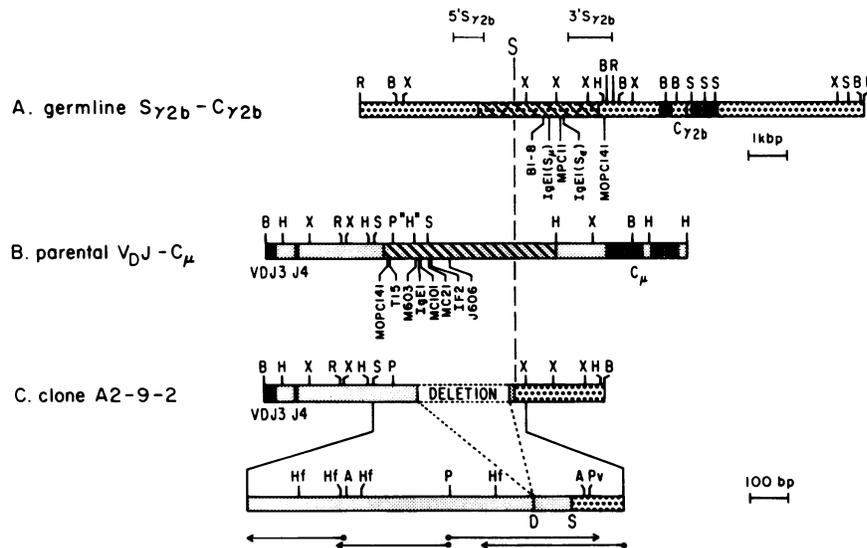

FIG. 2. Structure of the S$_\mu$-S$_{\gamma2b}$ rearrangement in the A2 line. (A) Partial restriction map of the genomic S$_{\gamma2b}$ region. (B) Partial restriction endonuclease map of the parental VDJ-C$_\mu$ region. (C) Partial restriction map of the BamHI insert fragment of clone 9-2-1 which was derived from genomic DNA of line A2 and contains the expressed V$_H$DJ$_H$ region and S$_{\gamma2b}$ sequences. The presence of the S$_\mu$ and S$_{\gamma2b}$ sequences in the noted regions of the clone has been confirmed by direct DNA sequence analysis (Fig. 3). Horizontal arrows represent sequencing strategy. The region noted D contains the point of intra-S$_\mu$ deletion. The vertical line labeled S indicates the recombination site between the S$_\mu$ and S$_{\gamma2b}$ regions. Cross-hatched areas correspond to switch regions, and blackened sites indicate C$_H$ exons. Previously reported switch recombination events in the S$_\mu$ and S$_{\gamma2b}$ regions are indicated in panels A and B by the names of the lines in which they occurred. This information was obtained from published sources (16, 18, 19, 21, 28–31, 36, 40). A 2.4-kb deletion within the S$_\mu$ region is also indicated in panel C. The location of the 0.8-kb 5'-S$_{\gamma2b}$ and 1.2-kb 3'-S$_{\gamma2b}$ probes are represented above panel A. R, EcoRI; B, BamHI; H, HindIII; S, SacI; X, XbaI; Hf, HinfI; A, AvaII; and P, PvuII.

probes when cleaved with BamHI (because the BamHI site lies between J_H2 and J_H3), and the productive allele will yield a fragment of similar size because the BamHI site lost in the V_HDJ_H3 join is restored by a similarly positioned BamHI site (relative to J_H3) in the V segment (Fig. 2A). BamHI-digested DNA from the A2 (γ_{2b}-producing) subclone revealed two novel fragments of 6.5 and 5.0 kb, which hybridized to the J_H probe (Fig. 1, lane F). The 6.5-kb fragment was shown to contain the productive V_HDJ_H based on hybridization to a probe specific for the expressed V_H gene (data not shown), whereas the smaller fragment presumably represented the J_H2-to-C_μ portion of the nonproductive allele. In the A2 subclone, both of these alleles contain large deletions in the J_H-to-C_μ intron (see below and reference 5). When assayed with the C_μ probe, BamHI-digested DNA from the A2 line revealed a novel fragment of 3.5 kb and a fragment of 5.0 kb which comigrated with the J_H-positive BamHI fragment corresponding to the nonexpressed allele, in addition to the 12.5-kb fragment corresponding to the 3' C_μ region (Fig. 1, lane C). This result suggested that a rearrangement event had unlinked the C_μ gene segment from the expressed V_HDJ_H segment in the A2 subclone and that this C_μ allele was retained in the cell in an altered context (i.e., the 3.5-kb BamHI fragment).

To determine whether the apparent rearrangement between J_H and C_μ on the productive allele of the A2 subclone was associated with the switch to γ_{2b} production in this line, we assayed BamHI-digested DNAs from the various lines for hybridization to a probe derived from the 3'-$S_{\gamma2b}$ region (see the legend to Fig. 2 for a description of the probe). Because the $S_{\gamma2b}$ switch region is contained within a 5.5-kb BamHI fragment, a class switch event involving fusion of the S_μ and $S_{\gamma2b}$ regions should generate a novel BamHI fragment which hybridizes to both the J_H and 3'-$S_{\gamma2b}$ probes. Although DNA from the parental 18-8 line contained only the 5.5-kb 3'-$S_{\gamma2b}$-positive BamHI fragment observed in liver DNA (Fig. 1, lanes G and H), DNA from the A2 subclone contained, in addition to a germ line fragment, a novel 6.5-kb 3'-$S_{\gamma2b}$-positive BamHI fragment which comigrated with the J_H-positive BamHI fragment found in that line (Fig. 1, compare lanes F and I). This result suggested that a rearrangement had occurred which brought the J_H and $S_{\gamma2b}$ of the expressed allele of A2 into close proximity. Additional support for this conclusion was obtained by similar blotting analyses with other enzymes (data not shown).

The class switch event in the A2 line involved recombination between S_μ and $S_{\gamma2b}$ regions. The nature of the potential class switch recombination event suggested from the restriction mapping analyses was further analyzed as follows. Partial MboI digests of genomic DNA from the A2 line were cloned into Charon 30, and clones were isolated which hybridized to both J_H- and 3'-$S_{\gamma2b}$-specific probes. A partial restriction endonuclease map of the internal 6.5-kb BamHI fragment (which comigrated with the J_H- and 3'-$S_{\gamma2b}$-positive BamHI fragment of the A2 line) of one such clone (A2-9-2) is presented in Fig. 2C. Comparison of the map of this clone to that of the embryonic $S_{\gamma2b}$-$C_{\gamma2b}$ region (Fig. 2A) and to that of the expressed V_HDJ_H-C_μ region of the 18-8 parental line (Fig. 2B) clearly suggests that the class switch in the 18-8 line occurred by a recombination-deletion mechanism which resulted in the fusion of the S_μ and $S_{\gamma2b}$ sequences with removal of the intervening C_H genes (including C_μ). We confirmed this interpretation by determining the nucleotide sequence of various regions of the clone to directly demonstrate the presence, at the indicated locations, of the expressed V_HDJ_H region of the parental 18-8 line (V_H81Y [5])

(data not shown), as well as the S_μ and $S_{\gamma2b}$ regions (see below).

A detailed analysis of the nucleotide sequence near the S_μ-to-$S_{\gamma2b}$ joint in clone A2-9-2 indicated that the expressed heavy-chain allele of the A2 subclone contained two rearrangement events within the S_μ region: an internal deletion (indicated as D in Fig. 2 and 3), and a joining event between the highly repetitive portion of the S_μ region and the $S_{\gamma2b}$ region (indicated as S in Fig. 2 and 3). Comparison of the sequence of clone A2-9-2 to that of the germ line S_μ region (Fig. 3, S_μ 5') allowed us to locate the 5' end of the deletion event within the S region (see the legend to Fig. 3). However, although a small portion of the S_μ region located near and very homologous to that of the 3' end of the deleted region has been sequenced (Fig. 3, S_μ 3' sequence), the highly repetitive nature of this portion of the S_μ region and the lack of extensive germ line sequence in this region preclude unequivocal identification of the exact germ line location of this end of the deletion. However, the unique repetitive structure of the A2-9-2 sequence 3' to the deletion point clearly identifies it as deriving from the highly repetitive portion of the S_μ region (28). For the same reason, we can also say that the switch recombination event occurred within the highly repetitive portion of the S_μ region; the location of this joint was ca. 100 bp downstream of the 3' end of the internal deletion (Fig. 3). The switch recombination point upstream of the γ_{2b} gene could be located (Fig. 2B) and occurred in the unique 49-bp repeat region that has previously been shown to be involved in switch recombination events in more mature cells (Fig. 3) (28). Four ambiguous bases, which could have been derived from either participating strand, occurred at the deletion joint, and one occurred at the switch recombination joint (Fig. 3, boxed sequences). Such ambiguous bases are frequently observed in D-to-J_H joints (35).

The A2 line retains two copies of the C_μ gene and two copies of upstream $S_{\gamma2b}$ sequences. Since switching occurred by the classical recombination-deletion mechanism in the 18-8 line, it was curious that this line apparently retained two copies of the C_μ gene (5). To confirm that the two distinct C_μ alleles occurred within the same cell, we assayed BamHI-digested genomic DNA from a series of subclones of the A2 line for hybridization to the C_μ probe. All of the subclones analyzed contained both the 5.0-kb C_μ-positive fragment which derived from the nonexpressed allele as well as the novel 3.5-kb C_μ-positive fragment which was unlinked from the J_H allele (Fig. 4).

The most interesting explanation for the retention of two C_μ copies in A2 would be the retention by the cell of the DNA segment lying within the switch recombination points (see below). Such a mechanism would predict the retention of $S_{\gamma2b}$ sequences just 5' to the recombination point but in a rearranged form. To test this possibility we assayed BamHI-digested DNAs from liver and A-2 cells for hybridization to a probe representing the region just upstream from the $S_{\gamma2b}$ switch recombination point (Fig. 2A, 5'-$S_{\gamma2b}$ probe). As indicated above, the 3'-$S_{\gamma2b}$ probe hybridized strongly to the 5.5-kb germ line BamHI fragment from the unrearranged allele and to the rearranged fragment of 6.5 kb which contained the expressed V_HDJ_H and the S_μ sequences (Fig. 5A). The 5'-$S_{\gamma2b}$ probe also hybridized to the 5.5-kb germ line fragment from the unrearranged allele and weakly to the rearranged 6.5-kb fragment due to some $S_{\gamma2b}$ region homology at its 3' end (Fig. 2A); however, it hybridized strongly to a unique 3.5-kb BamHI fragment (Fig. 5B). Analyses with other enzymes gave similar results (data not shown). These
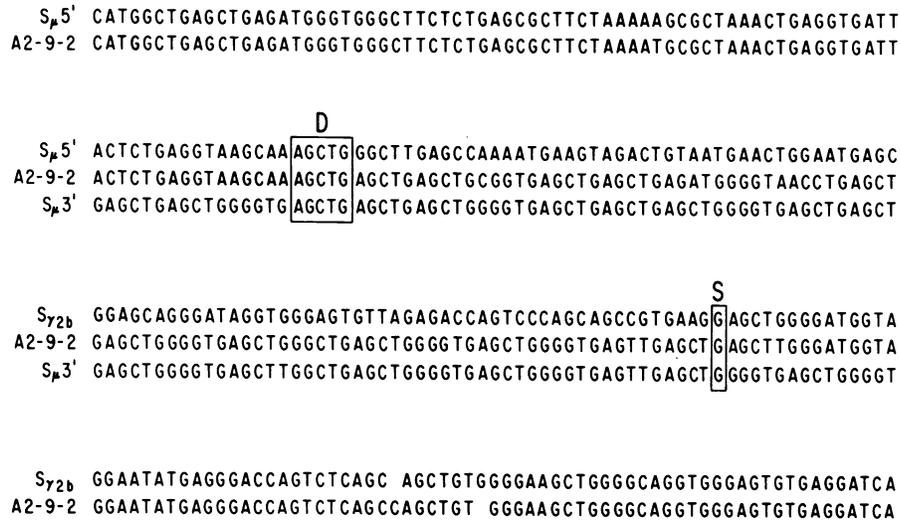
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S_μ5'  CATGGCTGAGCTGAGATGGGTGGGCTTCTCTGAGCGCTTCTAAAAAGCGCTAAACTGAGGTGATT
A2-9-2  CATGGCTGAGCTGAGATGGGTGGGCTTCTCTGAGCGCTTCTAAAATGCGCTAAACTGAGGTGATT


                                   D
S_μ5'  ACTCTGAGGTAAGCAA|AGCTG|GGCTTGAGCCAAAATGAAGTAGACTGTAATGAACTGGAATGAGC
A2-9-2  ACTCTGAGGTAAGCAA|AGCTG|AGCTGAGCTGCGGTGAGCTGAGCTGAGATGGGGTAACCTGAGCT
S_μ3'  GAGCTGAGCTGGGGTG|AGCTG|AGCTGAGCTGGGGTGAGCTGAGCTGAGCTGGGGTGAGCTGAGCT


                                                              S
S_γ2b  GGAGCAGGGATAGGTGGGAGTGTTAGAGACCAGTCCCAGCAGCCGTGAAG|G|AGCTGGGGATGGTA
A2-9-2  GAGCTGGGGTGAGCTGGGCTGAGCTGGGGTGAGCTGGGGTGAGTTGAGCT|G|AGCTTGGGGATGGTA
S_μ3'  GAGCTGGGGTGAGCTTGGCTGAGCTGGGGTGAGCTGGGGTGAGTTGAGCT|G|GGGTGAGCTGGGGT


S_γ2b  GGAATATGAGGGACCAGTCTCAGC AGCTGTGGGGAAGCTGGGGCAGGTGGGAGTGTGAGGATCA
A2-9-2  GGAATATGAGGGACCAGTCTCAGCCAGCTGT GGGAAGCTGGGGCAGGTGGGAGTGTGAGGATCA
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FIG. 3. The nucleotide sequence of S-region recombination events in the A2 line. The nucleotide sequences of the germ line $S_μ$ and $S_{γ2b}$ regions are compared with that of the corresponding region of the clone A2-9-2. The sequence of the A2-9-2 clone was determined by the method of Maxam and Gilbert by strategy outlined in Fig. 2C (expanded region). $S_μ$ 5' represents the germ line $S_μ$ sequence upstream from the $S_μ$ deletion (reproduced from *Nature* [36], with permission). Our sequence analysis of $S_μ$ actually extends ca. 1 kb 5' to the presented region and demonstrated nearly complete homology with that reported by Sakano et al. (36). $S_μ$ 3' refers to the germ line $S_μ$ sequence 3' to the site of the $S_μ$ deletion and 5' to the point of recombination with the $S_{γ2b}$ region (reproduced from *Nature* [28], with permission). Although the sequence 3' to the site of deletion and 5' to the site of recombination is clearly derived from $S_μ$, the highly repetitive nature of this portion of the $S_μ$ region precludes unequivocal identification of the exact germ line location of this portion of the clone. The $S_{γ2b}$ sequence (reproduced from the *Journal of Biological Chemistry* [29], with permission) is just 5' to the noted *Xba*I site. Recombination points D and S are described in the legend to Fig. 2.
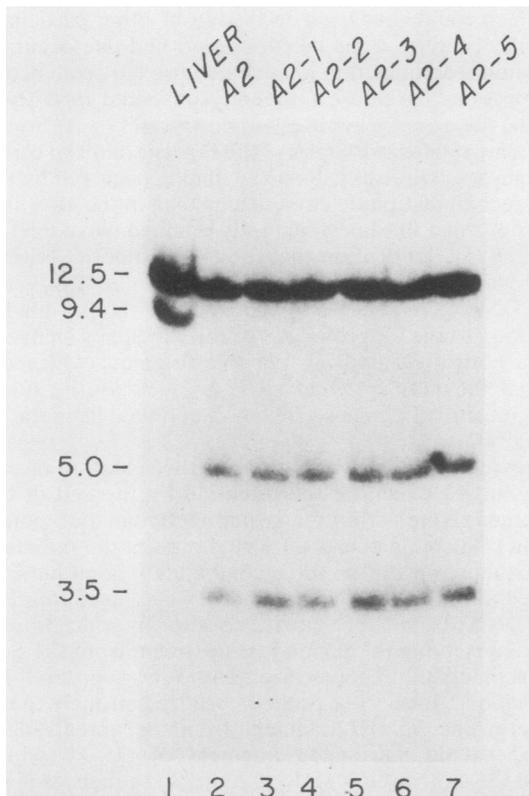

FIG. 4. Retention of two copies of the $C_μ$ gene in subclones of line A2. The 18-81 A2 line was subcloned by plating at limiting dilution in microtiter wells. DNAs from mouse liver, the parent A2 line, and various subclones were digested with *Bam*HI and analyzed for hybridization with the $C_μ$ probe as described in the legend to Fig. 1.

results strongly suggest that the $S_{γ2b}$ sequences upstream from the switch recombination point were also retained in the cell but in a rearranged form. Thus, both sequences just downstream from the $S_μ$ recombination point ($C_μ$) and just upstream from the $S_{γ2b}$ recombination point ($5'$-$S_{γ2b}$) appear to be retained in the cell in a rearranged form.

DISCUSSION

We demonstrated that the switch from μ to $γ_{2b}$ production by the A2 subclone of the 18-8 line is accompanied by a rearrangement event which juxtaposed the $γ_{2b}$ constant region gene segment to the expressed V_HDJ_H segment. This recombination event occurred between sequences lying within the previously defined $S_μ$ and $S_{γ2b}$ regions and was accompanied by the loss of the $C_μ$ (and other C_H) genes from the segment of the expressed chromosome lying between $S_μ$ and $S_{γ2b}$ but apparently not from the cell (see below). Both the mechanism of the switch in this line (recombination-deletion) and the fusion points of the switch recombination event (within S_H regions) are consistent with the classical recombination-deletion model for class switching that was derived by analyzing the heavy-chain gene structure of various plasmacytomas and hybridomas.

We feel that the class switch-related events that we have observed do not represent the low-frequency, homologous recombination-mediated events observed in myelomas (17, 33) for multiple reasons, including the following. (i) The frequency of switching in these A-MuLV transformants is very high in some isolates (5, 11). (ii) The class switch events occurred between the S region sequences, regions previously demonstrated to be involved in physiological class switch events. (iii) Like 18-8, all of the pre-B lines studied thus far have a predisposition to switch from μ to $γ_{2b}$ (see below), and yet the $S_μ$ and $S_{γ2b}$ regions are the most divergent S region sequences; low-frequency class switch variants of myelomas usually occur between (closely related) subclasses (17, 33). (iv) The 18-8 line and other

A-MuLV transformants which undergo class switch events also accumulate deletions in the S_μ region at high frequency (6; K. Kruger, M. G. Reth, G. D. Yancopoulos and F. W. Alt, manuscript in preparation); we now show that these deletions are within the same portion of the S_μ region that was involved in class switching. Significantly, more mature B-cell and T-cell lines do not appear to undergo this deletion activity during propagation (5; K. Kruger and F. W. Alt, unpublished data).

Recently DNA blotting analyses of class switch events in several other 18-8 derivatives (11) and in an independently derived A-MuLV transformant which had undergone a class switch event (1) suggested that switching in those lines was also mediated by a deletion mechanism. In both of these studies, class switch events were analyzed only by Southern blotting (1, 11). In the former study, the exact area of the class switch recombination event was not localized (11). In the latter study, blotting data suggested that the site of recombination was 5' to the $S_{\gamma 2b}$ region, leading to the suggestion that switching in pre-B cells may occur outside the S region (1); however, the exact nature of the recombination point and its relationship to S region sequences were not determined. Our studies, on the other hand, clearly indicate that cultured pre-B cell lines can undergo S region-mediated class switch recombination events. In confirmation of this conclusion, we have determined that a μ to γ_{2b} switch recombination event in an independent pre-B cell line (300-18) also occurred within the $S_{\gamma 2b}$ region (G. D. Yancopoulos, R. DePinho, and F. W. Alt, manuscript in preparation). It is notable that like A2, several other 18-8 derivatives or independently derived A-MuLV transformants switch from μ to γ_{2b} (8, 11; G.D. Yancopoulos and F. W. Alt, manuscript in preparation). The molecular basis and physiological relevance of the predisposition of A-MuLV transformants to switch from μ to γ_{2b} remain to be determined. Since our current studies clearly indicate that the switch occurs between S_μ and $S_{\gamma 2b}$ regions and since these are the most divergent S region sequences (28, 29), it seems possible that high-frequency switching to γ_{2b} in pre-B cells may be characteristic of that developmental stage.

We have observed retention of two copies of the C_μ gene in subclones of the γ_{2b}-producing A2 line. In this line, the C_μ gene on the nonproductive allele remains in its original configuration (linked to the nonproductive $V_H DJ_H$), but the C_μ gene which, presumably, was on the productive allele (the allele involved in the switch to γ_{2b}) is retained in the cell but in a rearranged configuration, i.e., unlinked to J_H (Fig. 1). Additional evidence for retention of the intervening sequences is provided by the retention of two copies of sequences just 5' to the $S_{\gamma 2b}$ switch recombination point, one of which was retained in a rearranged context (Fig. 5). Assuming that these retained, rearranged sequences are products of the same event, there are several likely mechanisms to explain their retention, including the following: (i) reintegration of the intervening DNA sequences which were excised as a result of S_μ-$S_{\gamma 2b}$ recombination occurring between two genes of the same chromosome, although it should be noted in the context of such a mechanism that the intervening DNA sequences that are excised as a result of intrachromosomal D-to-J_H or V_H-to-DJ_H recombination have never been observed to be retained by the cell (8); and (ii) retention in the cell as a result of class switch recombination between S_μ and $S_{\gamma 2b}$ sequences on sister chromatids or homologous chromosomes. Both sister chromatid and homologous chromosome recombination models have been invoked to explain class switch events (12, 15, 31), although
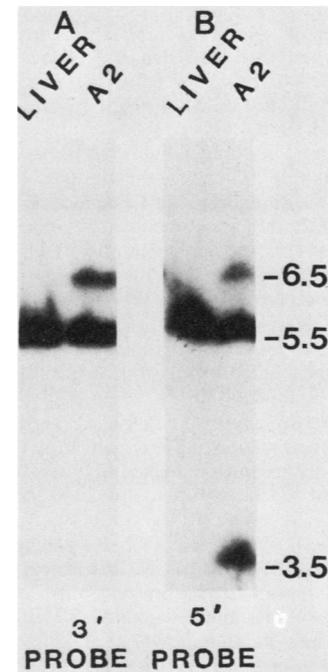

FIG. 5. Retention of $S_{\gamma 2b}$ sequences upstream of the recombination point. DNA from liver and the A-2 line was digested with BamHI, processed as indicated in the legend to Fig. 1, and assayed for hybridization to the 3'-$S_{\gamma 2b}$ (A) or 5'-$S_{\gamma 2b}$ (B) probes.

no direct evidence in support of either of these models is currently available. The retention of the intervening sequence is consistent with a class switch event involving a homologous chromosome exchange. Retained intervening sequences are not directly consistent with a sister chromatid exchange model but could have resulted from a previous class switch event involving sister chromatids as proposed to explain the retention of upstream sequences in cells that have undergone V_K-to-J_K joining of both chromosomes (41). We have observed retention of two copies of the C_μ gene in at least one other A-MuLV transformant that appears to have undergone a class switch event (K. Kruger, G. D. Yancopoulos and F. W. Alt, unpublished data), although deletion of the C_μ gene was observed to accompany class switch events in other A-MuLV transformants (1, 11; G. D. Yancopoulos, R. DePinho and F. W. Alt, manuscript in preparation). Although the physiological significance of the retained intervening sequences in γ_{2b}-producing A-MuLV transformants is presently unclear, resolution of the possible mechanisms underlying this phenomenon could provide valuable insight into the molecular details of the class switch process.

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