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On immunoglobulin heavy chain gene switching: two γ2b genes are rearranged via switch sequences in MPC-11 cells but only one is expressed

Rhonda B.Lang, Lawrence W.Stanton and Kenneth B.Marcu

Biochemistry Department and Molecular Biology Graduate Program, SUNY at Stony Brook, NY 11794, USA

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ABSTRACT

During B lymphocyte differentiation, switches in the expression of heavy chain immunoglobulin constant region (C_H) genes occur by a novel DNA recombination mechanism. We have investigated the requirements of the C_H gene switch by characterizing two rearranged γ2b genes from a γ2b producing mouse myeloma (MPC-11). One of the two γ2b genes is present in 2-3 copies per cell (γ2b strong hybridizer) while the other is present in ∿1 copy per cell (γ2b weak hybridizer). Genomic clones of the γ2b strongly hybridizing gene indicate that this is an abortive switch event between the $S_{\gamma 3}$ and $S_{\gamma 2b}$ regions. However, clones of the γ2b weakly hybridizing gene suggest a functional rearrangement due to the presence of V_H, J_H and S_μ sequences. The switch-recombination sites of these rearranged γ2b genes and those of other C_H genes show a high degree of preference for the sequence AGGTTG 5' of either the S_μ donor site or the appropriate C_H S acceptor site. AGGTTG and its analogs are rare in the S_μ region, are somewhat prevalent in S_α and in the case of S_μ are found 5' of a tandemly repeated DNA sequence (GAGCT, GGGGT) comprising most of S_μ.

INTRODUCTION

It is now a well accepted premise that a functional heavy chain immunoglobulin gene is created by two independent somatic DNA recombination events during B lymphocyte differentiation (1-3). The first recombination involves one of several hundred V_H genes, potentially one of a number of D_H mini-genetic elements and one of four J_H mini-genetic elements yielding a transcriptionally competent $VDJC_\mu$ gene (3,4). The second DNA recombination event involves a switch in expression from C_μ to one of six other C_H genes (γ3, γ1, γ2b, γ2a, ε or α) with no additional V_H gene rearrangements (2,3,5,6). C_δ would appear to be uniquely expressed by the differential RNA processing of a complex μ and δ transcription unit (7-9). The switch-recombination process may be manifested by sister chromatid exchanges which result in DNA deletions between C_H switch sites (6). A diagram of these events is shown in Figure 1.

A number of switch donor sites 5' of C_μ and switch acceptor sites 5' of the γ1, γ2b and α genes have been extensively characterized. In the case of

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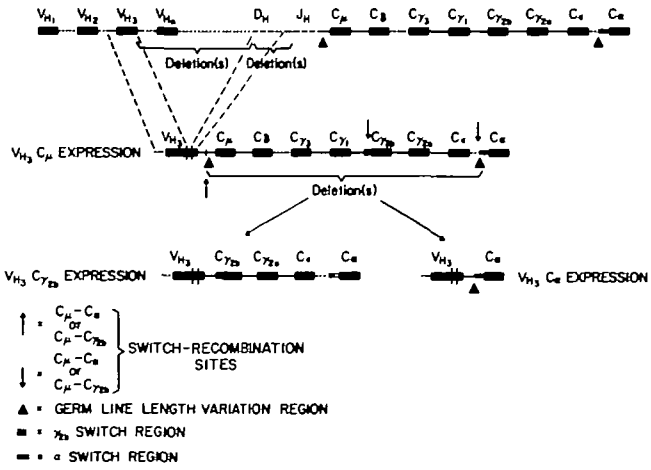

Figure 1. DNA recombination events associated with C_H gene expression.

two γ1 (2,5,6,10) and two α myelomas (5), tandemly repetitious DNA sequences located 5' of these C_H genes have been implicated in the switching mechanism and have been termed S regions (2). However, sequence analysis of the expressed γ2b gene of MOPC 141 has not revealed the presence of repetitive DNA sequences at the site of switch-recombination (3,11). The apparent lack of significant nucleotide sequence homology between the γ1 and α type acceptor S regions and their lack of homology with S_μ donor sites has prompted one group of investigators to propose a model for C_H class specific switch-recombinases (5). However, additional complications in the switch mechanism arise when one considers that no C_H class specificity appears to exist for determining the S_μ donor sites. Recently, more extensive DNA sequence analysis of the 5' flanking region of the germ line γ2b gene and a rearranged γ2b gene has revealed a rather homogeneous set of 49 base pair repeats which are located ~0.8-4.1 kbp 5' of the MOPC 141 γ2b gene recombination site (12). This repetitive sequence would appear to share extensive sequence similarities with other S_γ sequences and more limited homology with the shorter, tandemly repetitious sequences which define the majority of S_μ (12). These findings have led these workers to suggest that C_H gene switching may be a consequence of homologous recombination between short common sequences found 5' of all C_H genes (12).

We have previously shown that DNA sequences associated with or adjacent

to S_μ and S_α regions possess extensive sequence homology. These sequences delete during cloning in bacteria and undergo length variation in the germ lines of inbred and wild mice as well as DNA rearrangements in a variety of mouse myelomas (13,14). These 5' flanking C_μ and C_α sequences have been found to be highly conserved between mice and humans further implicating them as important components of the C_H gene switch (15,16).

In order to gain further insight into the molecular mechanism of the C_H gene switch, we have herein undertaken a study of the γ2b genes of the MPC-11 cell line (a γ2b producing Balb/c plasmacytoma) (17). MPC-11 has proven to be a most interesting cell line with regards to the stability and expression of both the light and heavy chain Ig polypeptides (18, 19). MPC-11 cells have been shown to spontaneously lose the ability to produce γ2b heavy chains at a rate of 1×10^{-3}/cell/generation (18,20) and also produce a unique light chain fragment mRNA (21-25). Upon mutagenesis these cells are capable of generating variants which produce either shorter γ2b chains (26), apparently complete γ2a chains (27-29) or γ2b-γ2a hybrid polypeptides (30,31). In this report, we describe the structures of two rearranged γ2b genes present in different copy numbers in MPC-11 cells. We provide formal evidence that recombination within S regions can yield both nonproductive as well as productive C_H genes suggesting a random selection process for C_H gene expression. Switch-recombination may be indirectly facilitated by short DNA sequences (GAGCT & GGGGT) which are a large portion of the S_μ repetitive region but a much smaller component of the $S_{\gamma 2b}$ repeat. However, the presence of a well conserved DNA sequence (AGGTTG) found either close to the 5' side of all C_H donor or acceptor switch sites and the general lack of conservation of the Balb/c $S_{\gamma 2b}$ repeat in a different mouse species suggests to us that other sequence specific components may also be necessary for proper C_H gene switching. The γ2b gene which we have identified as the expressed gene in MPC-11 cells has recently been observed to undergo a DNA rearrangement event in several γ2a producing MPC-11 switch variants derived by mutagenesis (32).

EXPERIMENTAL PROCEDURES

DNA Isolation, Cloning and Southern Blotting

DNA isolations, bacteriophage cloning, plasmid subcloning, restriction enzyme digestions and the preparation of radiolabeled probes for blotting experiments were all performed as previously described (13).

Southern transfers and genomic blot hybridizations were performed essentially as described (13,33). However, some genomic blot hybridizations were

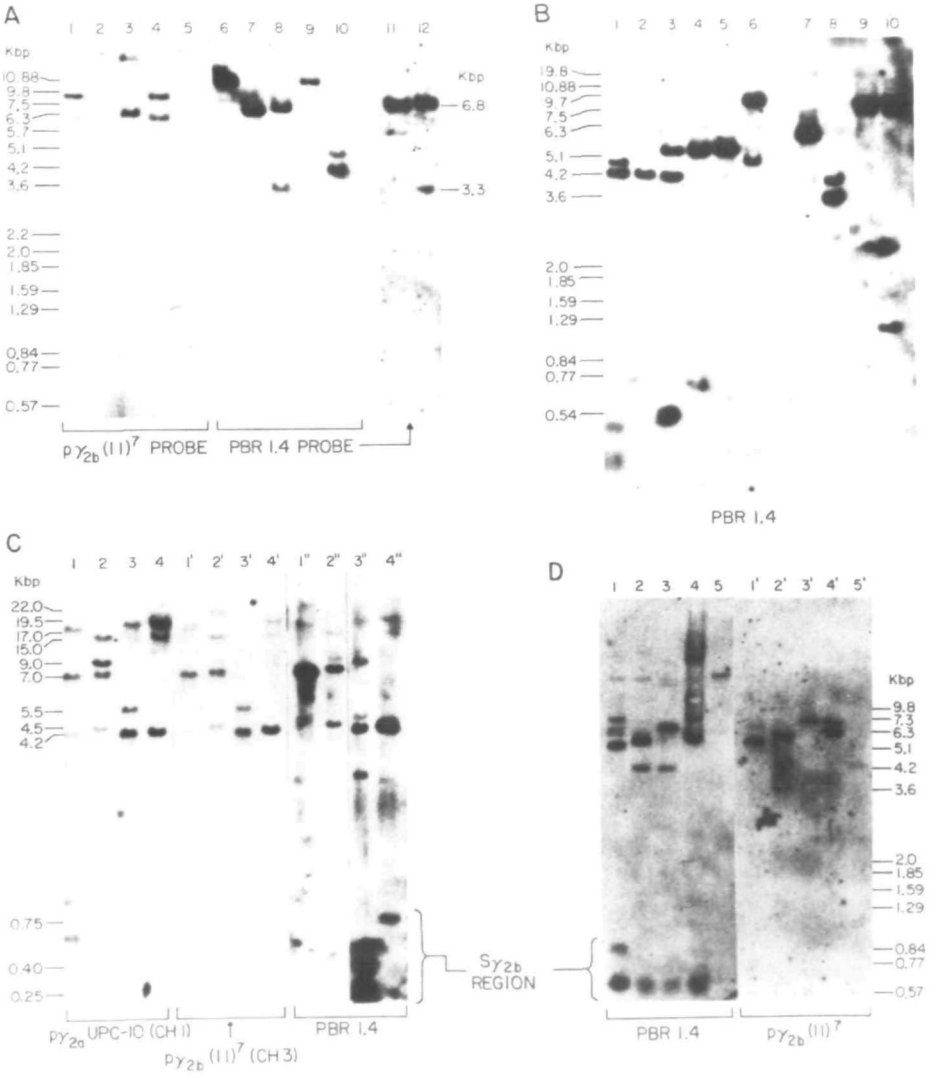

Figure 2. Comparative Restriction Digestions of MPC-11 and Mouse Liver DNAs Hybridized with γ2b, γ2a and $S_{\gamma 2b}$ Probes.

(A) Southern transfers of 0.8% agarose gel-fractionated Balb/c J liver DNA digested with HindIII (Lanes 1 & 6), SacI (Lanes 2 & 7) and EcoRI (Lane 11) or MPC-11 DNA digested with HindIII (Lanes 4 & 9), SacI (Lanes 5 & 10) and EcoRI (Lanes 3, 8 & 12) were either hybridized to pγ2b(11)^7 probe (Lanes 1-5) or pBR1.4 probe (Lanes 6-12). The weak 6.3 kbp HindIII band which hybridizes to the pγ2b(11)^7 probe in both Balb/c (Lane 1) and MPC-11 (Lane 4) samples

is due to the γ2a gene. A side by side comparison of the EcoRI fragments detected in Balb/c and MPC-11 DNAs by the pBR1.4 probe is provided in Lanes 11 and 12 to clearly indicate the presence of a unique, weakly hybridizing γ2b gene (∿3.2 kbp) in the MPC-11 sample. pBR1.4 positive bands of even weaker intensity in Lanes 6-9 are presumably due to cross-hybridization with other S_γ regions.

(B) Balb/c and MPC-11 DNAs were digested respectively with KpnI (Lanes 1 & 2), BglII (Lanes 3 & 4), BamHI (Lanes 5 & 6), SacI (Lanes 7 & 8) and HindIII (Lanes 9 & 10) and hybridized to pBR1.4.

(C) BglI and KpnI digestions of Balb/c DNA (Lanes 1, 1' and 1'' for BglI, Lanes 3 and 3' and 3'' for KpnI) and MPC-11 DNA (Lanes 2, 2' and 2'' for BglI and Lanes 4, 4' and 4'' for KpnI) were hybridized to either a γ2a C_H1 domain probe (Lanes 1-4). a γ2b C_H3 domain probe (Lanes 1'-4') or the $S_{\gamma 2b}$ probe, pBR1.4 (Lanes 1''-4''). The γ2a C_H1 probe was prepared by restriction digestion of a full length cDNA clone of the UPC-10 myeloma γ2a mRNA (52) and the γ2b C_H3 probe was obtained in a similar manner from the pγ2b(11)[7] sequence (37). Nitrocellulose filters were washed in 3xSSC 0.1% SDS for 1-2 hours prior to autoradiography.

(D) BglII digestions of liver DNAs of M.M. domesticus, Balb/c J, NZB, C57Bl/6J and M. pahari were hybridized to the pBR1.4 probe (Lanes 1-5) or the pγ2b(11)[7] probe (Lanes 1'-5') respectively. The intensely hybridizing bands in Lanes 1-4 correspond to portions of the $S_{\gamma 2b}$ repeat (see text) and the weaker bands of high molecular weight are due to S_γ cross-hybridization. The 3' $S_{\gamma 2b}$ fragments are shown to hybridize to the $C_{\gamma 2b}$ probe in Lanes 1'-4'. Only one high molecular weight band is observed with pBR1.4 for M. pahari DNA (Lane 5) while the M. pahari γ2b gene equivalent would appear to be a weak band of ∿4.3 kbp (Lane 5').

carried out at 65°C for 16-24 hours in a solution with the following ingredients: 3xSSC, 10% dextran sulphate (pharmacia), 0.1% each of polyvinylpyrrolidone 360, ficoll 400 and bovine serum albumin (Sigma Fraction V, #A-4503), 0.1% SDS, 5mM EDTA, 10 μg/ml poly rA, 50 μg/ml of sheared salmon sperm DNA and 1-2 x 10^6 cpm/ml of probe) (33) (B. Van Ness and P. Brodeur, personal communication). Stringent filter washes were performed as described subsequent to autoradiography with intensifier screens (13).

DNA Sequencing

DNA sequencing was performed on 0.3mm x 40cm or 90cm long gels (for fragments >250 bp in length) (34) essentially as described (35) with one slight modification. Piperidine was removed by ethanol precipitation as opposed to repeated lyophilizations in water. Briefly, piperidine was removed by precipitation in 0.3M NaAc pH 7.0 with 2.5 parts of 100% ethanol, precipitates were resuspended in 200 λ of 0.3M NaAc pH 7.0, reprecipitated and washed in 70% ethanol and finally dessicated prior to resuspension in formamide-dye mix

for loading sequencing gels. C and C+T reactions were resuspended and reprecipitated twice. Sequencing ladders were observed to consist of sharper bands allowing easier reading of the DNA sequences (M. Krystal, personal communication).

Genomic Southern Blots Reveal Multiple γ2b Gene Rearrangements in MPC-11 Cells

In order to obtain a clear, overall picture of the contextual arrangement of the γ2b and γ2a genes in MPC-11 cells, we initiated these studies by analyzing Southern blots of MPC-11 DNA with $C_{\gamma 2b}$ (22,37), $C_{\gamma}2a$ (52), $S_{\gamma 2b}$ and J_H DNA probes. Data obtained with SacI and BglI clearly demonstrated the presence of rearranged MPC-11 γ2b genes using either a $C_{\gamma 2b}$ probe ($p\gamma 2b(11)^7$) (23,37) or a γ2a C_H1 probe (52) with no maintenance of a germ line γ2b sequence (see Figures 2A and 2C). However, EcoRI, HindIII and BglII digestions gave no evidence of γ2b gene rearrangements (Figure 2A). These results indicate that the γ2b gene rearrangements in MPC-11 cells occur considerably 5' (i.e., at least 1.5 kbp) of the recombination site observed for the MOPC 141 γ2b gene which was found to be ∿1.0 kbp 5' of $C_{\gamma 2b}$ (3,11). Detailed restriction enzyme maps of the rearranged MPC-11 γ2b genes were prepared by resorting to a 5' $C_{\gamma 2b}$ switch region probe (see origin of pBR1.4 in Figure 6, genomic blot data in Figure 2A-C and γ2b gene restriction maps in Figure 3). These experiments clearly indicate that: (1) two types of rearranged γ2b genes are present in MPC-11 cells, (2) the γ2b genes exist in different copy numbers per cell giving rise to both strongly and weakly hybridizing bands unique to MPC-11 cells and (3) the sites of rearrangement are ∿2.5 kbp 5' of $C_{\gamma 2b}$ and probably less than 400 bp apart.

MPC-11 Cells Contain Only One Rearranged J_H Region

In order to attempt to determine which of the rearranged γ2b genes might be the sequence expressed in MPC-11 cells, we used two subcloned genomic sequences of the Balb/c germ line J_H region (i.e., pj_0 and pj_{11} in Figure 4) (13,14) as probes for our genomic blot studies. These experiments (see Figure 5A-C) allow us to draw the following conclusions: (1) no germ line J_H arrangement exists in MPC-11 cells, (2) only one rearranged J_H sequence exists in MPC-11 cells, and (3) HindIII and BamHI data both show that J_{H1} is deleted with J_{H2} presumably undergoing a recombination event with the MPC-11 V_H gene. Indeed, the nucleotide sequence of the V_H region of the MPC-11 γ2b mRNA has revealed that J_{H2} is used in the recombination event (39). When MPC-11 DNA is cut with BamHI, the pBR1.4 band corresponding to the γ2b weakly hybridizing gene and one of the J_H hybridizing bands are observed to co-migrate (Figure 2B

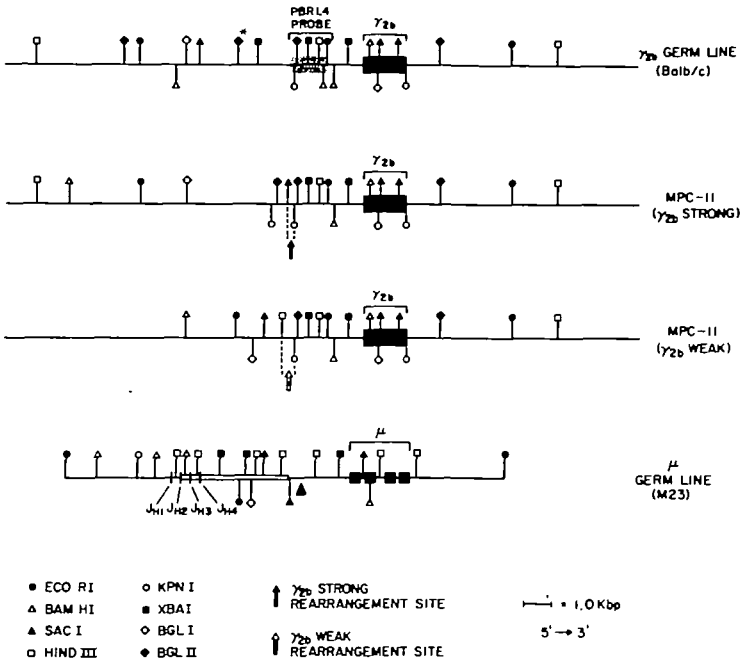

Figure 3. Genomic Restriction Maps of a γ2b Strongly Hybridizing Gene and A γ2b Weakly Hybridizing Gene in Comparison to Maps of the Balb/c Germ Line γ2b and μ genes.

and 5A). No J_H hybridizing bands appear to co-migrate with pBR1.4 bands attributable to the γ2b strongly hybridizing gene. Unfortunately, most of the restriction enzymes used (i.e., except for BamHI) are probably cutting

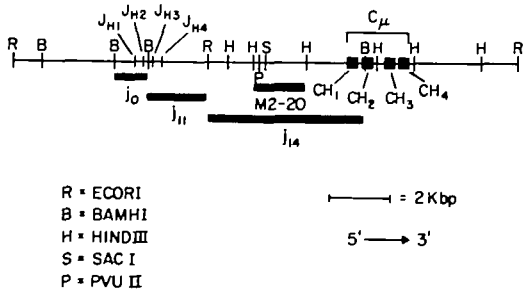

Figure 4. Restriction map of C_μ Flanking Sequences and Location of Subcloned hybridization probes. Restriction sites only relevant to the text are indicated.

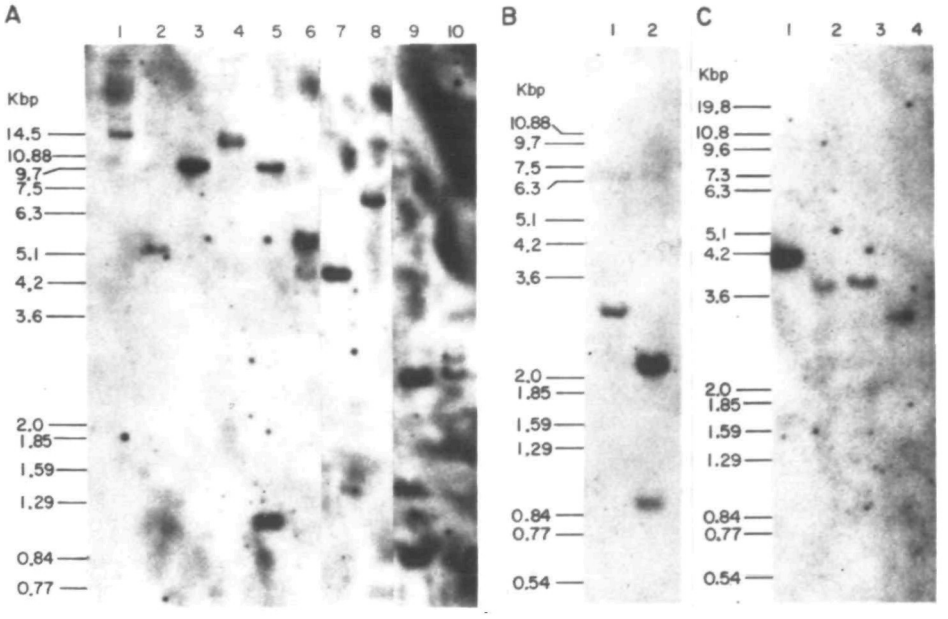

Figure 5. Southern transfers of restriction digestions of Balb/c J liver and MPC-11 DNAs hybridized to the entire Balb/c J_H region (pj_0 + pj_{11} probes in Figure 4).

(A) Balb/c J and MPC-11 DNAs digested with KpnI (Lanes 1 & 2), BglII (Lanes 3 & 4), BamHI (Lanes 5 & 6), SacI (Lanes 7 & 8) and HindIII (Lanes 9 & 10) respectively.

(B) MPC-11 DNA digested with EcoRI (Lane 1) and XbaI (Lane 2).

(C) Balb/c J and MPC-11 DNAs doubly digested with KpnI + BglII (Lanes 1 & 2) or KpnI + EcoRI (Lanes 3 & 4) respectively.

in between the pBR1.4 hybridizing region and the J_H region in the expressed γ2b gene. This conclusion is substantiated by an extensive analysis of γ2b genomic clones.

Isolation of Two Rearranged γ2b Genes from MPC-11 Cells: Genomic Clones of an Abortively Rearranged γ2b Gene

We then set about to obtain genomic clones corresponding to the two types of rearranged γ2b genes and initially screened a partial EcoRI library of MPC-11 DNA prepared in Charon 4A phage (40) with our γ2b gene specific probes. Two genomic clones containing overlapping sequences (M68 and C62 in Figure 6) were obtained with the pBR1.4 probe from ~$6x10^5$ phages of the unamplified MPC-11 gene library. The structures of this first set of clones completely

agree with the genomic restriction map of the γ2b strongly hybridizing gene. A comparison of the restriction map of the MPC-11 γ2b gene to those of both a γ2b and a γ3 gene isolated from a Balb/c sperm DNA library indicated that the 5' flanking sequences of the γ3 gene and those of the γ2b gene have recombined generating the MPC-11 γ2b strongly hybridizing gene (Figure 6). Hybridization experiments performed with the pBR1.4 probe, a 5' flanking γ3 gene probe (pγ3-2.7) and the rearranged EcoRI fragment present in the MPC-11 C62 clone (i.e., the C62-6.8 probe) all support the idea that this rearrangement was a result of a homologous DNA recombination event occurring between $S_{\gamma 3}$ and $S_{\gamma 2b}$ regions. Genomic Southern blot experiments performed with a γ3 gene probe (γ3-6.0) in addition to the above hybridization reagents show that the γ3 gene is deleted from MPC-11 DNA (data not shown). A comparison of sequences flanking the Balb/c γ3 gene (SL51 & SL14) with those of the γ2b strongly hybridizing gene of MPC-11 (C62 & M68) clearly indicates that at least ∿12 kbp of DNA 5' of the recombination site is directly 5' of the germ

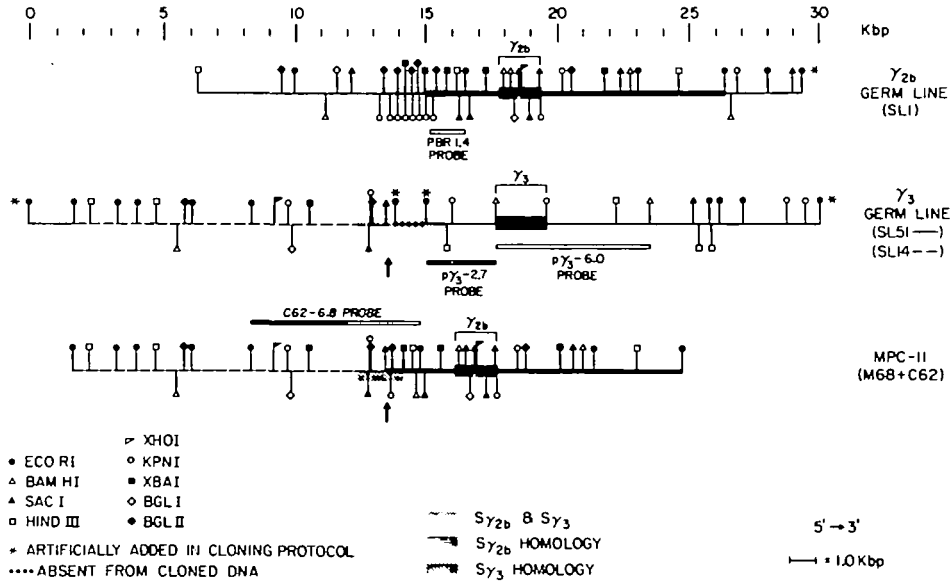

Figure 6. "$S_{\gamma 3}$ and $S_{\gamma 2b}$ recombination generates a non-functionally rearranged γ2b gene in MPC-11 cells." The pBR1.4 probe was originally obtained as a 1.4 kbp EcoRI fragment from a Balb/c mouse sperm γ2b genomic clone (SL59) and was subcloned into pBR322. A small portion (∿0.9 kbp) of the 5' flanking γ3 region (·····) was not obtained in our two γ3 clones (SL14 and SL51). These clones were linked by comparison with other published data (53).

line $\gamma 3$ gene. No sequences in our $\gamma 2b$ strongly hybridizing gene clones hybridize to either J_H or S_μ probes (data not shown). These results would strongly argue that this MPC-11 $\gamma 2b$ gene is an abortive rearrangement apparently due to a single recombination event between $S_{\gamma 3}$ and $S_{\gamma 2b}$. Our findings extend the observations of other workers who have isolated and determined the DNA sequence of the site of recombination of a rearranged $\gamma 2b$ gene in MPC-11 cells (12).

Genomic Clones of the Expressed $\gamma 2b$ Gene:

In order to obtain a clone corresponding to the MPC-11 $\gamma 2b$ weakly hybridizing gene, we screened $\sim 2 \times 10^6$ plaques of the MPC-11 library with our pj_{11} probe (Figure 4) (13,14) and obtained two positive clones. Upon plaque purification, both of these clones were also found to hybridize to our pj_0, pBR1.4, $p\gamma 2b(11)$[7] and S_μ probes as anticipated for a functionally rearranged $\gamma 2b$ gene. Restriction digestions indicated that these clones are identical. A complete restriction map of the expressed $\gamma 2b$ genomic clone (RBL 216) is shown in comparison to M23 (a germ line Balb/c μ gene clone) and SL1 (a germ line Balb/c $\gamma 2b$ gene clone) in Figure 9.

Southern blots performed with the pj_0 and pj_{11} probes (13,14) allowed us to position the location of the MPC-11 V_H gene in RBL 216 (see Figure 7A & B). As anticipated from our genomic Southern blots J_{H2} is utilized by the MPC-11 V_H gene. We confirmed that the MPC-11 V_H sequence was involved in this recombination event by comparing the pattern of restriction sites 5' of J_{H2} in RBL 216 with those anticipated from the nucleotide sequence of an MPC-11 V_H cDNA clone (39) (see Figure 9). This experiment was performed by purifying a 2.1 kbp HindIII fragment from RBL 216 which was expected to contain the V_H region, J_{H2}, and J_{H3} as well as some additional 5' and 3' flanking sequences. Since BamHI cuts between J_{H2} and J_{H3} (13,14) and our pj_0 probe ($J_{H1\ \&\ 2}$) can only hybridize 5' of this BamHI site, double digestions performed on this 2.1 kbp HindIII piece with BamHI and other enzymes allowed us to specifically map restriction sites 5' of this BamHI site by hybridizing with our pj_0 probe. These results confirm that the V_H sequence of the MPC-11 $\gamma 2b$ mRNA is 5' of J_{H2} in this $\gamma 2b$ genomic clone (RBL 216) which corresponds to our $\gamma 2b$ weakly hybridizing gene.

Southern blots performed with S_μ (pj_{14} and $pM2_{20}$ in Figure 4) and $S_{\gamma 2b}$ (pBR1.4) probes allowed us to position the switch-recombination site in this expressed $\gamma 2b$ genomic clone. This switch event occurred between the 5' HindIII site of the $M2_{20}$ sequence (S_μ) and the solitary KpnI site in pBR1.4 ($S_{\gamma 2b}$) (see Figures 4, 7C and 8A&B). Most of the S_μ region is deleted in the

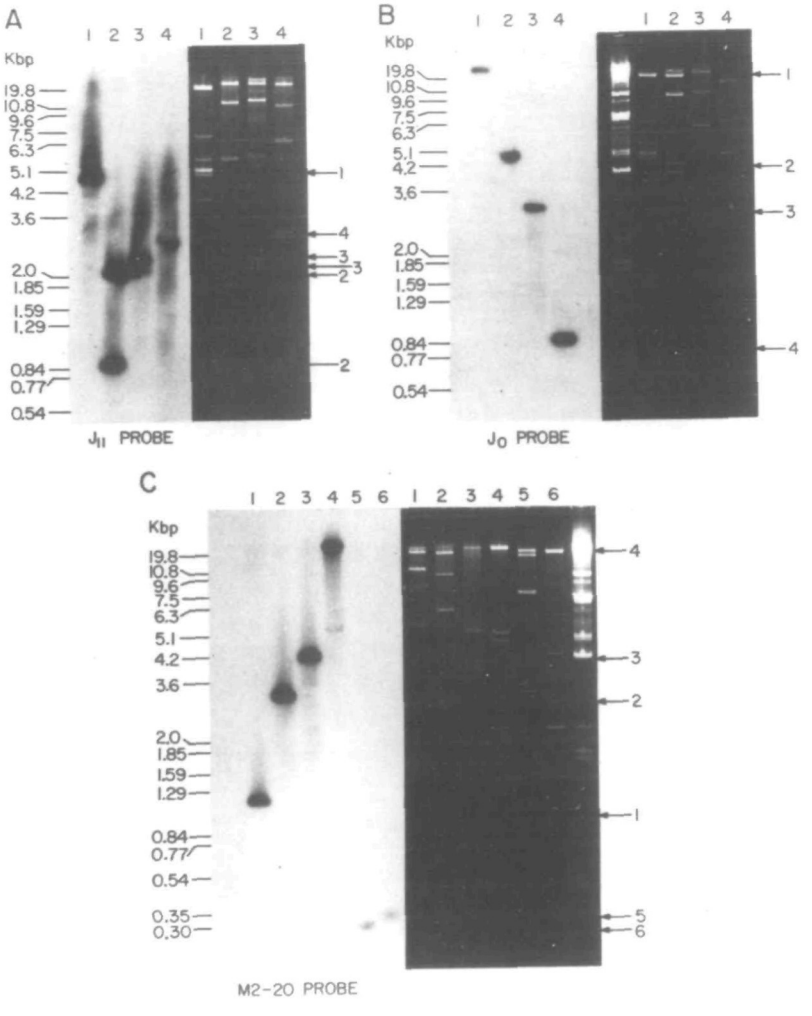

Figure 7. Analysis of Southern transfers of restriction enzyme digestions of the expressed γ2b genomic clone (RBL 216).

(A) RBL 216 DNA digested with BamHI, XbaI, HindIII and EcoRI (Lanes 1-4 respectively) hybridized to the pj_{11} probe.

(B) RBL 216 DNA digested with BamHI, KpnI, EcoRI and BamHI + EcoRI (Lanes 1-4 respectively) hybridized to the pj_0 probe.

(C) RBL 216 DNA digested with HindIII, EcoRI, SacI, BglII, KpnI + HindIII and BglII + HindIII (Lanes 1-6 respectively) hybridized to the $pM2_{20}$ probe. The origins of the probes are shown in Figures 4 and 6. Pictures of the ethidium stained gels are shown for comparison.

switch as shown by the markedly reduced hybridization response of the HindIII-KpnI recombination fragment with the $M2_{20}$ probe as compared to the $M2_{20}$ sequence itself (see Figure 8A).

S_μ Switches Directly to $S_{\gamma 2b}$ to Express the MPC-11 γ2b Gene

DNA sequence analysis (35) was performed on a portion of the subcloned pBR1.4 sequence and on the 275 bp HindIII-KpnI recombination piece within RBL 216 (see Figure 9 for sequencing strategies). These sequences are shown in comparison to the S_μ region (3) to precisely position the RBL 216 recombination site for the expressed gene. As shown in Figure 10, the μ↦γ2b switch occurred 6 nucleotides 5' of the KpnI site within our pBR1.4 probe and after this point, the sequence is essentially in complete agreement with

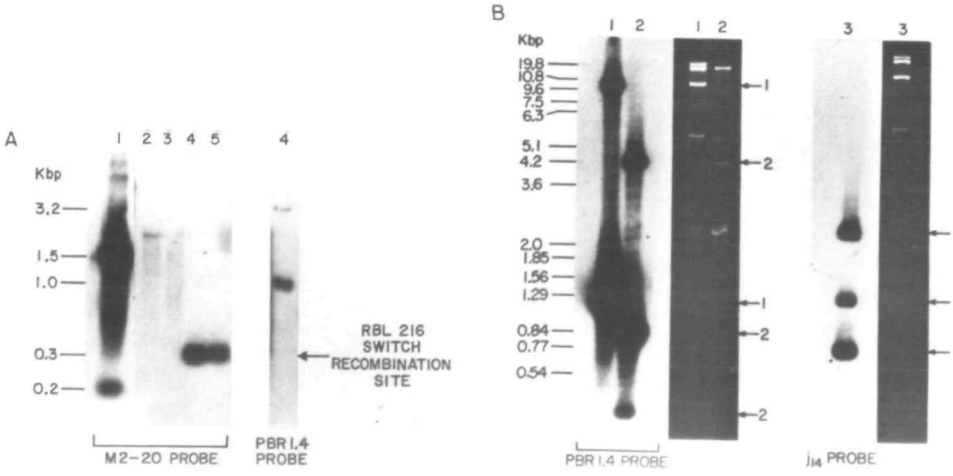

Figure 8. Restriction Digestion Analysis of the Expressed γ2b Gene (RBL 216) Recombination Site.

(A) Southern transfers of $pM2_{20}$ insert digested with PvuII (Lane 1), pBR1.4 digested with KpnI + EcoRI (Lane 2) or PvuII + EcoRI (Lane 3) and RBL 216 DNA digested with either KpnI + HindIII (Lanes 4 & 4') or KpnI + HindIII + PvuII (Lane 5) were hybridized to either $pM2_{20}$ probe (Lane 1-5) or pBR1.4 probe (Lane 4'). Weakly hybridizing bands in Lanes 2 and 3 are due to contaminating pBR322 sequences in the $M2_{20}$ insert probe recognizing their homologous sequences in the pBR1.4 plasmid.

(B) RBL 216 digested with HindIII (Lanes 1 & 3) or HindIII + BglII was hybridized to either pBR1.4 (Lanes 1 & 2) or pj_{14} (Lane 3). The pBR1.4 blot is somewhat overexposed to allow one to clearly discern the 325 bp recombination fragment in Lane 2. The overexposed bands are 1.3 kbp and 0.80 kbp in Lanes 1 and 2 respectively. Profiles of ethidium stained gels are shown for comparison.

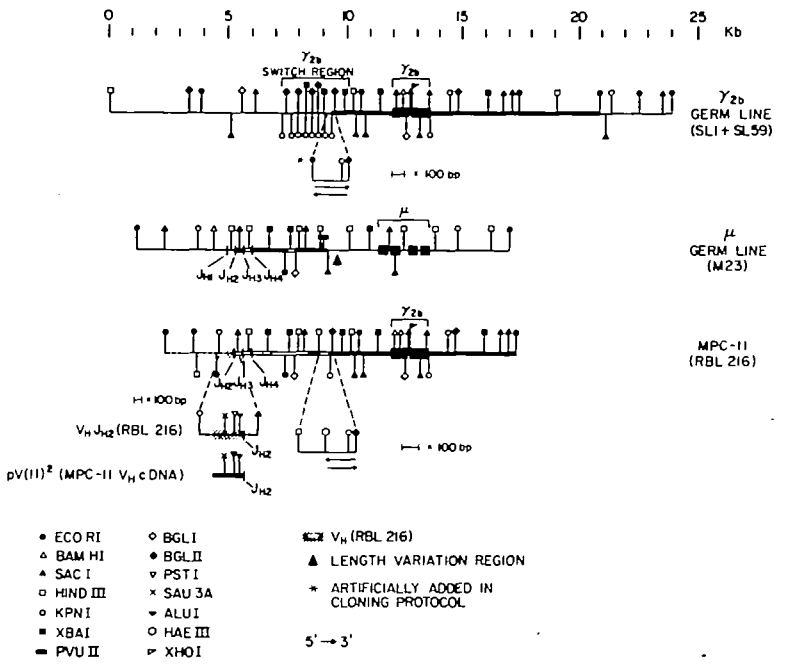

Figure 9. Restriction Maps of the Functiona-lly Rearranged MPC-11 γ2b Gene, and Balb/c Germ Line γ2b and μ Genes. Arrows indicate the DNA sequencing strategies performed on portions of the cloned DNAs. The region of the γ2b germ line DNA chosen for DNA sequencing corresponds to the 5' 235 nucleotides of the pBR1.4 sequence. pBR1.4 is a subclone of a 1.4 kbp EcoRI fragment originally obtained from a Balb/c sperm γ2b genomic clone, SL59. The restriction sites of the MPC-11 V_H cDNA clone, $pv(11)^2$, were obtained from the published DNA sequence (39).

the corresponding S_μ region (3). Relative to the S_μ region, the site of recombination is 52 nucleotides 5' of the SacI site in $M2_{20}$ (Fig. 4). This is considerably 5' of the highly repetitious, genetically unstable sequence component within S_μ (3,13,14,41).

MPC-11 Cells Possess Other Rearranged C_H Genes

We have found evidence for at least two types of rearranged γ2a genes (see Figure 2C), two rearranged α genes (14) and one rearranged μ gene (14) (Greenberg, R. and Marcu, K., in preparation) in MPC-11 cells. DNA rearrangement events have occurred both 5' and 3' of the MPC-11 γ2a genes (see Figure 2C). BglI appears to cut in both C_H hinge regions (36,42,43) but no where in between the genes thereby generating a large 17 kbp DNA fragment con-

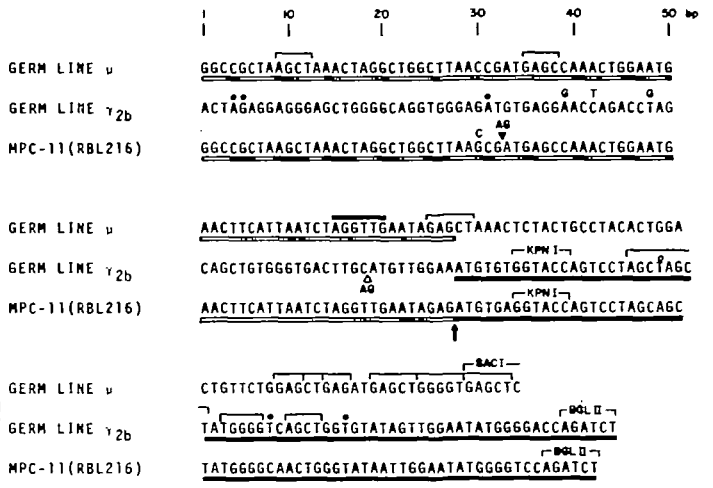

Figure 10. DNA sequences around the vicinity of the RBL 216 recombination site are compared to corresponding regions of the germ line μ and γ2b sequences. Short common sequences are indicated by brackets, a prevalent sequence (AGGTTG) uniquely found 5' of this and other switch sites is denoted by two solid overhead lines (═══). A sequence possessing a 5/6 match with AGGTTG (i.e., ATGTTG) is also seen four nucleotides 5' of the $S_{\gamma 2b}$ site. The independent $S_{\gamma 2b}$ site of the non-functional MPC-11 γ2b gene is exactly 251 nucleotides 5' of this functional $S_{\gamma 2b}$ site (see Figure 11) (12). Differences with other published work (12) are indicated above and below the sequences; nucleotides with overhead circles are single base insertions present in our sequence, nucleotides below the sequences are insertions present in other published work, the AG dinucleotide above line three is an insertion in our sequence in comparison to line one. The μ germ line sequence is taken from other published data (3).

taining part of the coding regions of both genes and all their intervening DNA. As expected, this 17 kbp fragment in Balb/c DNA was observed to uniquely hybridize with a γ2b C_H3 probe, a γ2a C_H1 probe and to an $S_{\gamma 2b}$ probe (pBR1.4) as shown in Figure 2C. The $S_{\gamma 2b}$ probe weakly hybridizes to this 17 kbp fragment presumably due to homology with $S_{\gamma 2a}$ sequences. Upon BglI digestion of MPC-11 DNA, four unique bands not present in the Balb/c control are observed to hybridize to both γ2a C_H1 and pBR1.4 probes. The lower two bands correspond to the two rearranged γ2b genes (Figure 3) and the weaker hybridization of the upper two bands with pBR1.4 suggests that these are γ2a genes. These rearranged γ2a genes are also clearly visible as two high molecular weight bands upon KpnI digestion.

DISCUSSION

We have shown that the γ2b expressing MPC-11 myeloma possesses two γ2b genes which have rearranged via switch-recombination sequences. One gene, which has rearranged via an $S_{\mu} \rightarrow S_{\gamma 2b}$ event contains the appropriate V_H and J_H sequences for the MPC-11 γ2b mRNA (39) while the other gene involved an $S_{\gamma 3} \rightarrow S_{\gamma 2b}$ event with no evidence for the involvement of S_{μ}. The overall size of our genomic clone corresponding to the functionally rearranged γ2b gene (i.e., RBL 216 in Figure 9) is approximately 10 kbp from the beginning of the V_H gene to the end of the $C_{\gamma 2b}$ gene agreeing well with an estimated size of 11.5 kb for the high molecular weight initial transcription unit of the MPC-11 γ2b gene (22). The functionally rearranged gene would also appear to possess a lower copy number per cell in comparison to the non-functional γ2b gene (i.e., 1 as opposed to 2-3). This is not difficult to understand when one considers that MPC-11 cells are polyploid containing ~65 chromosomes (Margulies, D.H., unpublished results).

A rearranged γ2b gene corresponding to our γ2b strongly hybridizing gene (unexpressed) has been found to be created by a recombination event within a set of homologous, 49 bp, repetitious DNA sequences located 5' of the γ3 and γ2b genes (12). Since these authors only observed one γ2b gene in Southern blots of MPC-11 DNA, they assumed this to be the functional γ2b gene and that the DNA recombination event had occurred between repetitive S_{γ} regions (12). As shown in Figure 6, neither V_H, J_H nor S_{μ} sequences are found 12 kbp 5' of our γ2b strongly hybridizing gene clones. It is known that MPC-11 cells are somewhat unstable and will lose the ability to produce heavy chains with a frequency of 10^{-3}/cell/generation (18,20). Unfortunately, it is not known whether this is indicative of either chromosome instability in these polyploid cells, γ2b gene deletion or a defect in gene expression (20). However, MPC-11 cells which have been repeatedly passaged in tissue culture give reproducibly low yields of γ2b mRNA (44) (Marcu, K. and Valbuena, O., unpublished results). In agreement with the results of this paper, an MPC-11 γ2b gene was recently detected by Southern blotting which shares some features in common with our functionally rearranged γ2b genomic clone (RBL 216) (45).

What are the Precise Molecular Requirements for C_H Gene Switching?

Unlike V_L-J_L or V_H-D_H-J_H recombination events which appear to require two small blocks of evolutionarily conserved nucleotides, that are separated by a defined length of spacer DNA, close to the site of recombination (3,4, 46,47), C_H gene switch-recombination has not been found to require a specific recombination site. This may simply be a reflection of the accuracy required for

proper C_H switching which occurs within the intervening sequences 5' of C_H genes and therefore does not create new coding information as in V_L or V_H gene formation. However, it has been proposed that C_H switching is either facilitated by homologous recombination between short, tandemly repetitious DNA sequences located 5' of all C_H genes (2,12,41,48) or by C_H class specific recombinases which recognize different repetitive sequences unique to individual C_H genes (5).

A comparison of the S_μ recombination site used by the MPC-11 γ2b gene with the S_μ donor sites and some of the S acceptor sites of other rearranged C_H genes is shown in Figure 11. We have termed these switch regions Donor (S_μ) and Acceptor (all other S_H regions) to emphasize that all functional switch events initiate in the S_μ region. The precise S_μ recombination sites of all these C_H genes are different. The only common feature is the presence of a well conserved short sequence (AGGTTG) positioned either 5' of the S_μ switch-recombination donor (D) site or adjacent to the appropriate C_H S acceptor (A) site. This sequence is perfectly conserved within 8 and 11 nucleotides 5' of the S_μ sites in MPC-11 and MOPC 603 (5) respectively. A

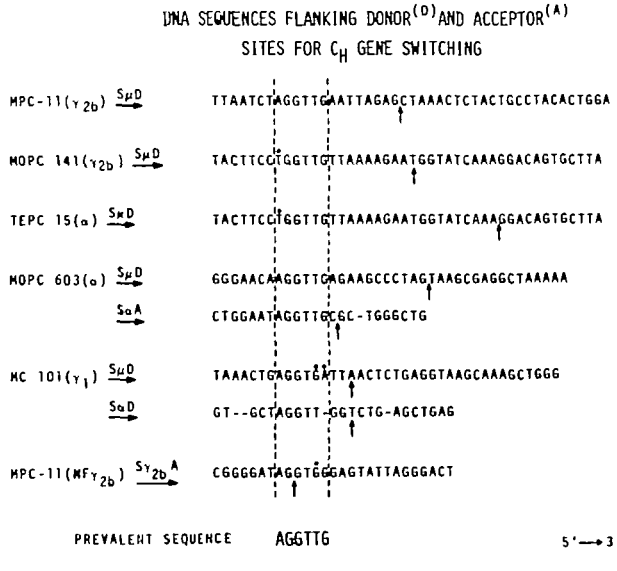

Figure 11. Switch sites in the vicinity of the S_μ region (donor switch region) and switch sites in the vicinity of the S_α and $S_{\gamma 2b}$ regions (acceptor switch regions) are compared and shown to possess a prevalent sequence 5' of the switch-recombination sites. Arrows indicate the sites of recombination. MOPC 141 (3,11), MOPC 603 and TEPC 15 (5), MC101 (5,6), MPC-11 non-functional (NF) γ2b gene (12), MPC-11 γ2b (Figure 10 of this paper).

sequence agreeing in 5 out of 6 positions is present 10 and 19 nucleotides 5' of the S_μ sites in MOPC 141 (3,11) and TEPC 15 (5) respectively. The weakest match is for the MOPC 101 γ1 gene (6) in which only 4 nucleotides of the sequence are present 5 nucleotides 5' of the S_μ site. However, a perfect match for the sequence is found 2 nucleotides 5' of the S_α donor sequence which actually switches to $S_{\gamma 1}$ in MC-101 (6). As indicated above, the S_μ site utilized by the direct switch to S_α in TEPC 15 is located farthest from this prevalent sequence (i.e., 19 nucleotides) but two sequences with 5 out of 6 base pairs in agreement with AGGTTG are also found 13 nucleotides 5' and 2 nucleotides 3' of the TEPC 15 S_α acceptor site (5). This sequence is not observed 5' of the S_μ site used in a direct switch to the C_α gene in the Balb/c J558 myeloma but a 5/6 match with AGGTTG is seen 2 nucleotides 5' of the S_α acceptor site (Harris, L. and Marcu, K., unpublished results). Interestingly, a similar sequence constitutes the $S_{\gamma 2b}$ recombination site in the non-functional MPC-11 γ2b strong gene (see Figure 11) (12). In addition, the S_μ site utilized for the switch to C_ε in an IgE hybridoma would appear to be quite close to the S_μ site of the MOPC 603 C_α gene (41).

Surprisingly, the sequence AGGTTG is not prevalent in the S_μ region. A search of the available S_μ sequences (3,41) (Greenberg, R. and Marcu, K., unpublished results) indicates that the AGGTTG sequence and its close analogues are quite rare in the entire S_μ region. In 1400 bp 5' of the MPC-11 S_μ site, this sequence occurs twice with a 6/6 match, three times with a 5/6 match and four times with a 4/6 match and five functional C_H switches occur 3' of four of these fifteen sequences. We also found only two 4/6 matches for this sequence within the entire ∿3 kbp of the genetically unstable, tandemly repetitious portion of the Balb/c S_μ region which basically only consists of two predominant short sequences, GAGCT and GGGGT (14,41) (Greenberg, R. and Marcu, K., unpublished results). Two 5/6 matched sequences also occur relatively close to the HindIII site which defines the 3' end of the length variation region within the $M2_{20}$ sequence (see Figure 3) (3,14). However, sequences like AGGTTG are most evident in the S_α region. Four 6/6 perfect matches and twenty-three 5/6 matches (with twenty of them as the sequence AGGCTG) occur randomly throughout 1.4 kbp of the S_α region (5). One is tempted to draw an interesting analogy between this sequence specific element which may play a role in C_H switching and the bacterial Chi sequence ($^{5'}$GCTGGTGG$^{3'}$) which mediates generalized recombination events over considerable distances (49,50). A possible role for Chi like sequences in recombination events associated with C_H gene expression has recently been suggested

(51).

In addition to the proposed involvement of the sequence specific element described here, repeated sequences within S regions share short common sequences which may indirectly facilitate C_H switching by homologous recombination (2,41). The largest repeated S region sequence constitutes the majority of the S_μ region which contains >3 kbp of DNA that almost solely consists of the short sequences GAGCT and GGGGT (41) (Greenberg, R. and Marcu, K., unpublished results). This S_μ region shares extensive sequence homology with all of S_α (12,14), the 3' portion of $S_{\gamma 3}$ (Stanton, L. and Marcu, K., unpublished results) and the majority of S_ε (Honjo, T., personal communication). However, overall homology of S_μ with 5' $S_{\gamma 3}$, $S_{\gamma 1}$, $S_{\gamma 2a}$ is much less substantial and completely lacking in the μ-δ intragenic region (Marcu, K., unpublished results). Another strong argument for the importance of this highly repetitive portion of the S_μ region for C_H switching is its conservation in other mouse species (13,14) and in humans (15,16). Evolutionary conservation arguments for the function of repetitive S_γ regions are not as convincing as for S_μ. As shown in Figure 2D, the $S_{\gamma 2b}$ repeat as revealed by BglII digestion appears to be quite similar in different inbred mouse strains and wild mice but quite different in an unrelated Asian mouse species (M. pahari).

In summary, we believe that a unique, short sequence (AGGTTG and its minor variants) may be a new, biologically significant, recognition element for C_H switching because: (A) AGGTTG or its close analogues are found adjacent to either the donor or acceptor sites of all C_H switches thus far determined, (B) this sequence does not closely resemble a class of short, common sequences (GAGCT, GGGGT) found within the repetitive portions of C_H S regions which have been proposed to mediate S-S recombination by homologous recombination (12), (C) except for the complex MOPC 603 α switch event (5) all the S_μ sites for other C_H genes are thus far clustered considerably 5' of the tandemly repetitious sequence component of the S_μ region (largely consisting of GAGCT and GGGGT) suggesting an indirect involvement of these short, common sequences in C_H switching. We cannot as yet disprove the other likely possibility that the repetitive S_μ region constitutes the primary switch region and all other events are secondary deletion phenomena associated with C_H gene switching. In any event, even these unusual secondary deletion events would then appear to have a strong preference for the sequence AGGTTG near the sites of recombination. Clearly, additional C_H switch sites need to be analyzed to either confirm or negate these and other suppositions concerning the precise molecular requirements for the C_H gene switch.

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