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A switch region inversion contributes to the aberrant rearrangement of a μ immunoglobulin heavy chain gene in MPC-11 cells

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Abstract

We describe the unique features of an aberrantly rearranged μ immunoglobulin heavy chain gene isolated from MPC-11 cells (a γ_{2b} producing Balb/c plasmacytoma). A novel rearrangement has occurred 1.5 Kb 5' of the MPC-11 μ gene (denoted 18bμ) resulting in the deletion of the majority of the repetitive switch region (S_μ) and 5' flanking DNA including the Joining (J_H) sequences. The remainder (275 bp) of the S_μ repeat has undergone a complete sequence inversion. DNA sequences 5' of the inverted S_μ sequence do not resemble Variable (V_H), Diversity (D), J_H or their conserved flanking sequences. A DNA sequence localized 5' of the inverted S_μ sequence, (p18bμ-1.4) detects a small family of homologous sequences in Balb/c DNA. The 18bμ-1.4 like sequences lack homology to S_μ, exhibit flanking sequence polymorphisms in 5 out of 6 inbred mouse strains and undergo partial or complete deletion in 5 out of 10 plasmacytomas tested. Two 18bμ-1.4 homologous sequences display a higher copy number in C57Bl/6, AL/N and CAL9 mouse strains.

INTRODUCTION

DNA deletions contribute to the efficient expression of immunoglobulin heavy chain constant region (C_H) genes in terminally differentiated B lymphocytes (1). In mouse plasmacytomas and hybridomas, a switch in expression from C_μ to one of six other C_H genes (i.e., γ_3, γ_1, γ_{2b}, γ_{2a}, ε or α) results in the deletion of all C_H genes 5' of the expressed C_H isotype (2-6). A class of repetitive DNA sequences localized 1.5 Kb 5' of all C_H genes with the exception of C_μ have been implicated in the C_H class switch and defined as switch (S) regions (7-12). Switch regions consist of two types of common sequences ($(GAGCT)_nGGGGT$ and YAGGTTG) which are believed to mediate switching by homologous recombination (10,11). C_H switches result from the direct recombination of S_μ and a downstream S_H region or by successive switches initiating from S_μ (8,11-14). C_H switching is believed to be mediated by either sister chromatid exchanges (14) or intramolecular deletions (1-4). Aberrant rearrangements are generated by

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defective interswitch region recombination (15) or by the recombination of S regions with DNA sequences which lie outside of the C_H gene locus (16-18). In this report, we describe a novel aberrant rearrangement occurring 5' of the μ gene in the MPC-11 myeloma. The 5' flanking J_H elements and most of S_μ are deleted leaving the remainder of S_μ in an inverted orientation. A member of a small family of non-C_H gene associated DNA sequences, which exhibits a high level of genetic instability in plasmacytomas and inbred mouse strains, has recombined with the inverted S_μ sequence.

METHODS

Molecular cloning and Southern blot hybridizations (19) were all performed as described (15,20,21). DNA fragments were subcloned into pBR322 and sequenced according to Maxam and Gilbert (22).

RESULTS

Cloning and DNA Sequence Analysis of an Aberrant μ Gene: A case of Switch Region Inversion.

MPC-11 cells possess multiple rearranged C_H genes (14,18). A single copy of a functionally rearranged γ_{2b} gene is present due to a direct $S_\mu \rightarrow S_{\gamma 2b}$ switch (15). Multiple copies of an aberrant γ_{2b} gene (generated by an abortive $S_{\gamma 3}$ x $S_{\gamma 2b}$ recombination) reside on unexpressed C_H chromosomes (9,15). Rearranged μ and α genes also exist in the MPC-11 genome (4,23) and may reside on the same inactive C_H chromosome as the aberrant γ_{2b} gene. Since MPC-11 cells only contain one J_H region which is linked to the expressed γ_{2b} gene (15), we decided to determine the origin of the aberrant μ gene which lacks 5' flanking J_H sequences.

A restriction endonuclease map of a genomic clone (18bμ) of the aberrant MPC-11 μ gene is presented in Figure 1. Southern hybridizations were performed on 18bμ with J_H, S_μ and C_μ probes (20). As summarized in Figure 1, a DNA rearrangement occurring ∿1.5 Kb 5' of the C_μ gene in the 18bμ clone contributes to the deletion of the four J_H sequences and 3' flanking DNA including the majority of S_μ. However, a portion of the repetitive S_μ sequence remains in the 18bμ clone.

DNA sequences at the site of recombination were subcloned into pBR322 for further study. A 2.6 Kb BamHI fragment (18bμ-2.6 in Fig. 1) contains the rearranged S_μ region and the 5' portion of C_μ in its germ

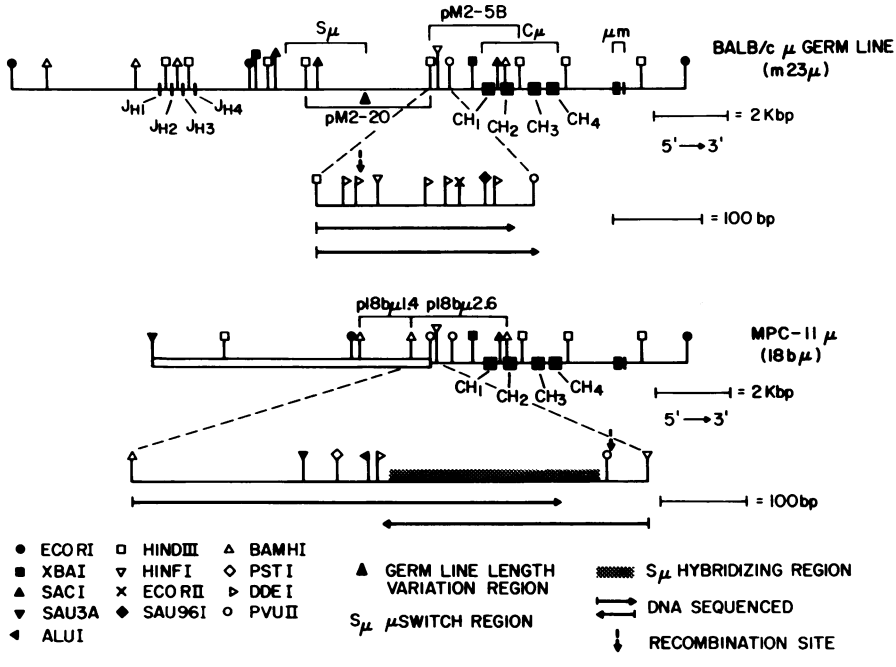

Figure 1.

Restriction endonuclease maps of genomic clones of MPC-11 μ gene (18bμ) and Balb/c μ gene (M2). 18bμ was isolated from a partial Sau 3A library of MPC-11 DNA inserted in Charon 28 phage (31). Fine structure restriction mapping and DNA sequencing (22) were performed on subcloned restriction fragments pl8bμ- 2.6 and pM2-5B from the aberrant MPC-11 μ gene (18bμ) and a Balb/c germ line gene (M2) (20) respectively. The sequencing strategies are indicated and the sequences are displayed in Figure 2.

line context. The nucleotide sequence of ∿600 bp at the 5' end of 18bμ-2.6 is shown in Figure 2 in comparison to the corresponding germ line DNA sequence 5' of the Balb/c C_μ gene. Remarkably, the entire S_μ hybridizing region within 18bμ-2.6 consists of a 275 bp inverted S_μ like sequence. An additional deletion event has also occurred beyond the 3' boundary of the germ line repetitive S_μ region (defined by HindIII fragment pM2-20 in Figure 1) bringing this unusual rearrangement closer to the C_μ gene. The nucleotide sequence upstream of the inverted S_μ like region bears no obvious resemblance to V_H, D, J_H or their flanking putative recombination sequences (CACTGTG and GGTTTTTGT or their inverse complements CACAGTG and ACAAAAACC) (13,24,25).

The origins of both the inverted S_μ like sequence and its 5' flanking region were explored by comparative Southern hybridization of

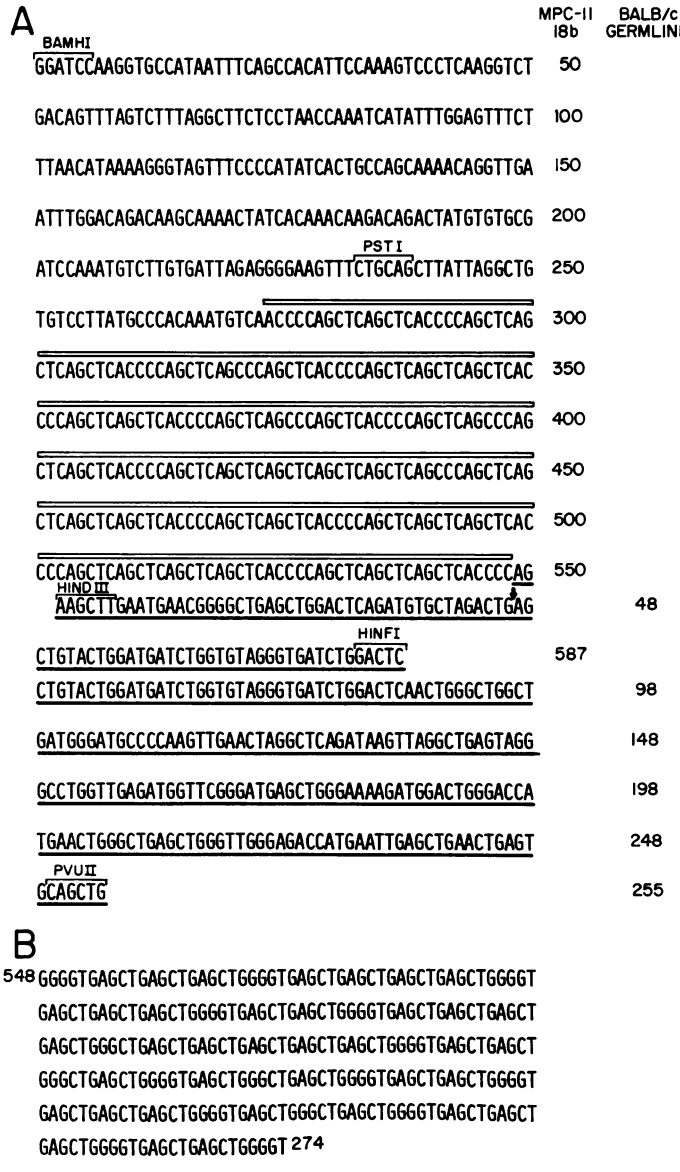

Figure 2.

A) DNA sequence of 587 bp of 5' end of pl8b$_\mu$-2.6 and 255 bp at 5' end of pM2-5B. Balb/c germ line sequence (pM2-5B) and its counterpart in pl8b$_\mu$-2.6 are indicated by a filled-in bar. The site of recombination in the MPC-11 μ gene is indicated by an arrow.

B) DNA sequence marked by an overhead open bar in panel A displayed in an inverted orientation. This sequence is identical to a portion of the germ line S_μ region (10).

Balb/c and MPC-11 DNAs with DNA probes flanking the 18bμ recombination site. A 1.4 Kb BamHI fragment 5' of the rearrangement site (i.e., p18bμ -1.4) detects a small family of homologous sequences in either EcoRI or HindIII digested Balb/c DNA (see Fig. 3). We will refer to these bands as the non-C_H gene associated 18bμ-1.4 sequence family. All members of the 18bμ-1.4 family are qualitatively retained in MPC-11 DNA. New bands of 9.6 Kb (EcoRI) and 8.0 Kb (HindIII) are present in MPC-11 DNA and correspond to the cloned 18bμ gene (see Figure 3A). Several 18bμ-1.4 homologous sequences present in Balb/c DNA appear to be reduced in intensity in MPC-11 DNA. The loss of these sequences from the MPC-11 genome are probably related to the formation of the 18bμ gene. However, this will only be formally proven upon cloning and characterizing the germ line 18bμ-1.4 sequence. The predominant 18bμ-1.4 bands in MPC-11 and Balb/c DNAs are also observed with the 3' adjacent 18bμ-2.6 sequence as shown in Figures 3B and 4A. Predominant bands unique to 18bμ-2.6 are evident upon BamHI (Fig. 3B) and HindIII (Fig. 4A) digestion of Balb/c

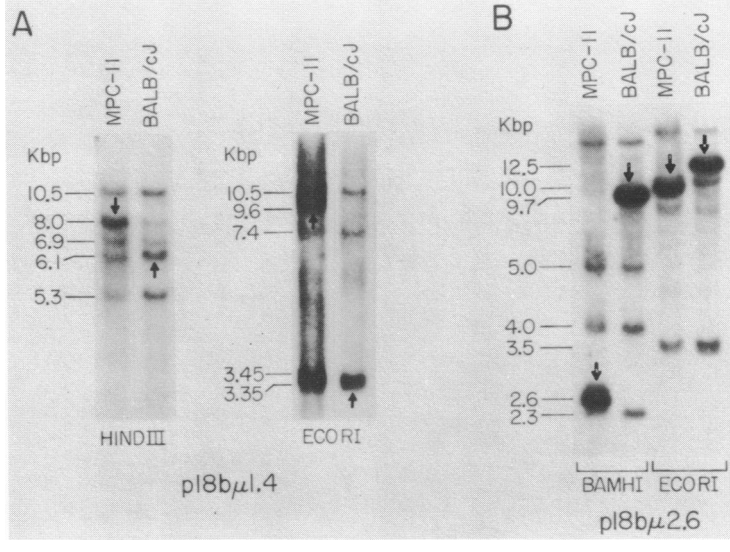

Figure 3A and B.

Southern hybridization (19) of p18bμ-1.4 and p18bμ-2.6 sequences to restriction endonuclease digested Balb/c and MPC-11 DNAs. Hybridizations were performed as described (15,21). Arrows in the MPC-11 lanes of Panel A indicates the 18bμ gene. Arrows in the Balb/c lanes of Panel A indicate the position of a band which is diminished in intensity in MPC-11 DNA. Arrows in panel B indicate the 18bμ sequence (MPC-11 lanes) or the germ line μ gene (Balb/c lanes).

DNA and represent the germ line C_μ and S_μ regions. The size of the major BamHI fragment detected by the 18bμ-2.6 probe in MPC-11 is identical in size to the 18bμ-2.6 sequence itself demonstrating that no significant rearrangements have occurred during the cloning of the 18bμ gene.

Hybridization experiments performed with the 18bμ-2.6 sequence and a germ line S_μ probe (pM2-20) (19) confirm that the inverted S_μ like sequence in 18bμ is truly derived from the S_μ region (see Fig. 4). The 18bμ-2.6 probe detects two major HindIII bands in Balb/c DNA (2.2 Kb and 3.7 Kb) in addition to the bands observed with the 5' adjacent 18bμ-1.4 probe. The 2.2 Kb band contains the first two C_μ domains and 5' flanking DNA while the 3.7 Kb band corresponds to the 5' adjacent S_μ region (20,23). The only major band detected by our S_μ probe is the 3.7 Kb S_μ HindIII fragment. None of the 18bμ-1.4 positive HindIII bands significantly hybridize to the S_μ probe. Therefore, the inverted S_μ like sequence directly arises from the S_μ region, and is not found adjacent to the 18bμ-1.4 sequence in the Balb/c germ line. DNA sequences 5' and 3' of the S_μ inversion suggest that the limits of the sequence inversion maybe

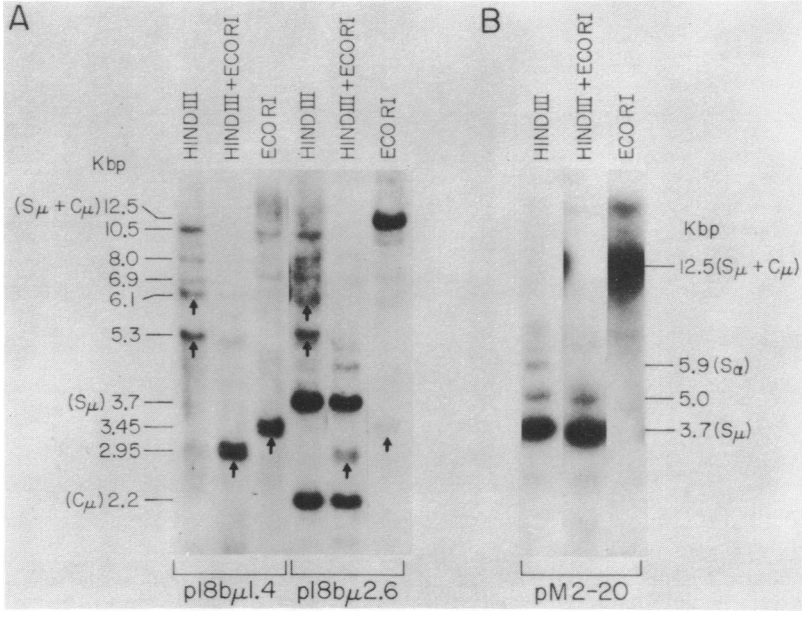

Figure 4.
Southern hybridization of p18bμ-1.4; p18bμ-2.6 and pM2-20 (S_μ) probes to restriction endonuclease digested Balb/c DNA. Bands indicated by arrows are detected by both 18bμ probes.

within the boundaries of the repetitive S_μ sequence (See Figure 2).

18bμ-1.4 Detects A Polymorphic, Genetically Unstable DNA Sequence Family

We next explored the genetic properties of the 18bμ-1.4 sequence family. Surprisingly, all members of the 18bμ-1.4 family display a high degree of flanking sequence polymorphism amongst different inbred mouse strains as shown in Figure 5. Five out of six strains tested possess one or multiple differences in the 18bμ-1.4 family. CBA and C3H/HEJ mice have identical 18bμ-1.4 patterns. This later result is not unexpected since these strains have identical C_H allotypes and V_H haplotypes (26, Brodeur & Riblet, personal communication). One member of the 18bμ-1.4 family displays a genetic polymorphism in AL/N and Balb/c mice. CAL9 mice (a Balb/c congenic of AL/N allotype) only contain the Al/N type 18bμ-1.4 sequences (see pattern Figure 5). This result strongly suggests that at least one member of the 18bμ-1.4 family is present on the Ig heavy chain chromosome #12. Flanking sequence polymorphisms (akin to those observed for the 18bμ-1.4 sequences) have also been described for

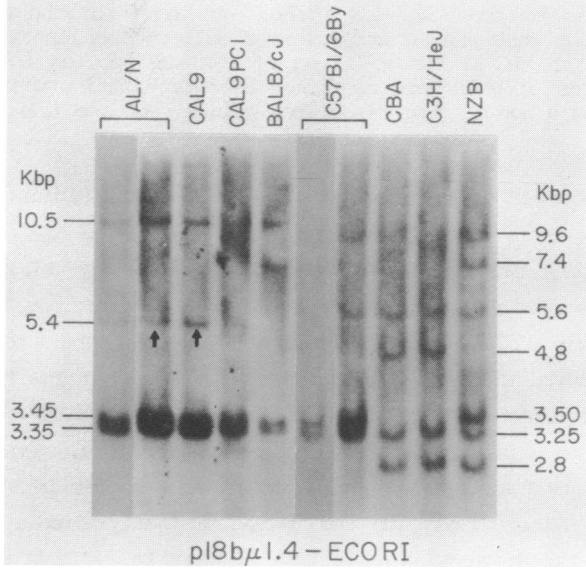

Figure 5.
Southern hybridization of EcoRI digested AL/N, CAL9, CAL9-PC1, Balb/cJ C57Bl/6By, CBA, C3H/HeJ and NZB DNAs to the 18bμ-1.4 probe. Arrows indicate a 5.4 Kb AL/N specific band that appears in both AL/N and CAL9 DNAs but is absent in Balb/c DNA. Two exposures (1x and 4x) are shown for the AL/N and C57Bl/6By lanes to allow for the visualization of the 3.35/3.45 EcoRI doublet. 7.5 μg of DNA was digested in all cases.

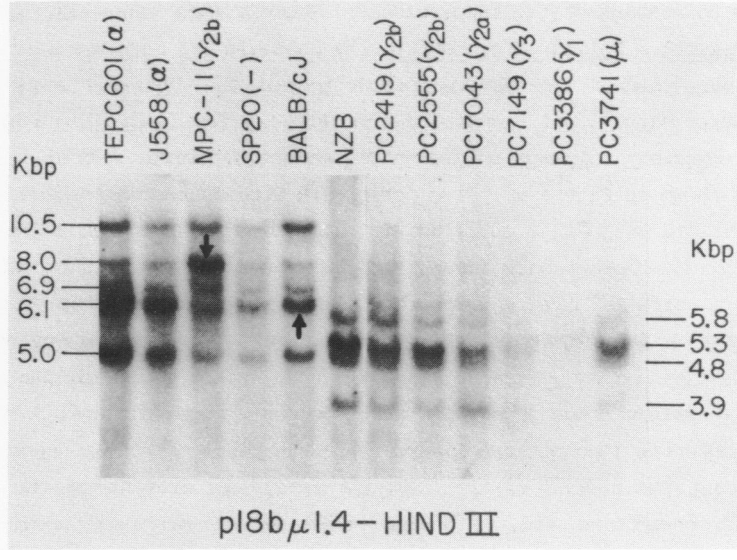

Figure 6.
Southern hybridization of HindIII digested Balb/c, NZB and plasmacytoma DNAs to the 18bμ-1.4 probe. An arrow in the MPC-11 lane indicates the 18bμ gene and an arrow in the Balb/c lane identifies a band which is weaker in MPC-11 DNA. 7.5 μg of DNA was applied to each lane. Similar quantities of DNAs were confirmed by hybridization with C_H switch region probes and a non C_H gene associated unique sequence (17).

V_H and D sequences in inbred mice (27,28, Brodeur and Riblet, personal communication).

Interestingly, two members of the 18bμ-1.4 family (3.35 Kb and 3.45 Kb EcoRI bands in Figure 5) are present in higher copy number in AL/N and possibly C57Bl/6 mice (i.e., ∿3-5 fold higher than other strains). As shown in Figure 5, the increased band intensity of two 18bμ-1.4 like sequences in AL/N is also observed in the CAL9 strain and in CAL9 PC1 (a γ_2b producing plasmacytoma). Therefore, two 18bμ-1.4 homologous sequences are clearly increased in copy number in strains of AL/N allotype. Minor bands of the 18bμ-1.4 family display similar intensities and therefore comparable copy numbers in all inbred strains tested (see Figure 5). More extensive analysis will be required to understand the significance of this genetic phenomenon.

Five out of ten plasmacytomas 18bμ-1.4 like sequences (see Figure 5). Two NZB plasmacytomas (PC7149 and PC3386) retain barely detectable levels of these polymorphic sequences. SP2-O cells (a non Ig producing

variant of a γ_2b, producing myeloma) (29) contain reduced quantities of 18bμ-1.4 sequences compared to a Balb/c liver control. The partial or complete loss of one 18bμ-1.4 like sequence appears to be coupled to the loss of other 18bμ-1.4 sequences possibly implying their genetic linkage. However, no significant deletions of 18bμ-1.4 homologous sequences are observed in three Balb/c and two NZB plasmacytomas.

DISCUSSION

The 18bμ gene represents the first instance of an S_μ region associated sequence inversion. A DNA inversion has also recently been observed in the case of an aberrant D-J_H recombination in an Abelson virus transformed pre-B cell line (30). The later D-J_H inversion was proposed to result from multiple recombination events between the conserved heptamer and nanomer sequences flanking D and J_H elements (30). The 18bμ gene would also appear to derive from several recombinations: 1) a member of a non-C_H gene associated sequence family (defined by the 18bμ-1.4 sequence) has recombined with the S_μ region, thereby deleting the majority of the repetitive S_μ sequence and 5' flanking DNA, 2) a remaining portion of the repetitive S_μ region has undergone an inversion within the boundaries of the S_μ repeat and 3) a deletion event has occurred between the 3' boundary of the repetitive S_μ region and the C_μ gene. Alternatively, the deletion and inversion of S_μ may be coupled to each other and the polymorphic non-C_H associated DNA may have recombined subsequent to the S_μ inversion. Since the repetitive portion of S_μ is intimately involved in the 18bμ rearrangement, it is tempting to consider this event a genetic property of the S_μ region. Inversions within S_μ could contribute to the known genetic instability of the S region (20,23). The S_μ inversion may be explicable by a series of recombination events within S_μ but cannot be satisfactorally explained by sister chromatid exchanges since the inversion event would require a minimum of two breaks in the same DNA molecule. The aberrant S_μ structure of the 18bμ gene may have prevented C_H switching from S_μ thereby avoiding deletion of C_μ. This notion seems plausible given that an S_μ mediated rearrangement has deleted the downstream γ_3 and γ_1 genes from the MPC-11 genome (15) in the absence of S_μ participation.

The loss of 18bμ-1.4 like sequences may be a consequence of V-D-J recombinations. The MPC-11 18bμ rearrangement may have occurred subsequent to an abortive D-J recombination (25) on the unexpressed C_H

chromosome. This idea would imply that the 18bμ-1.4 family lies 5' of the D elements in the mouse germ line. However, the absence of any obvious deletions in the 18bμ-1.4 family in three Balb/c plasmacytomas (TEPC601, J558 and MPC-11) and the apparent elevated copy number of two 18bμ-1.4 like sequences in AL/N mice would collectively suggest that 18bμ-1.4 sequences may be involved in more complex genetic phenomena such as unequal sister chromatid exchanges. Further studies on the context of 18bμ-1.4 sequences in recombinant inbred mouse strains will allow us to precisely determine their genomic location relative to V_H, D and J_H sequences.

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