Volume 10 Number 19 1982 Nucleic Acids Research

Nucleotide sequence and properties of the murine γ_3 immunoglobulin heavy chain gene switch region: implications for successive C_γ gene switching

Lawrence W.Stanton and Kenneth B.Marcu

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

Received 7 June 1982; Revised and Accepted 6 August 1982

Abstract

During B lymphocyte differentiation, immunoglobulin heavy chain constant region (C_H) genes undergo a unique series of DNA recombination events culminating in the C_H class switch. C_H switch (S) regions are located 2 kb 5' of each C_H gene except δ (i.e. μ, $\gamma 3$, $\gamma 1$, $\gamma 2b$, $\gamma 2a$, ϵ and α). We describe the structural features of the γ_3 switch region. Hybridization experiments show that $S_{\gamma 3}$ has remarkable homology to both S_μ and other S_γ regions while S_μ possesses limited homology to the other S_γ sequences. However, S_μ possesses extensive sequence homology with S_ϵ and S_α. The nucleotide sequence of $S_{\gamma 3}$ reveals higher densities of S_μ repetitive sequences (GAGCT and GGGGT) and another S region common sequence (YAGGTTG) than observed for $S_{\gamma 1}$, $S_{\gamma 2b}$ or $S_{\gamma 2a}$. In addition, the conservation of S_μ like repetitive sequences in S_γ regions is correlated with the 5'→3' γ gene order (i.e. $S_{\gamma 3} > S_{\gamma 1} > S_{\gamma 2b} > S_{\gamma 2a}$). A model is presented which suggests that the unique features of $S_{\gamma 3}$ may allow for successive switches from C_μ to any C_γ gene.

INTRODUCTION

Switches in the expression of immunoglobulin heavy chain constant regions (C_H) occur during B lymphocyte differentiation (1-3). A unique series of DNA recombination events which result in the deletion of C_H genes have been implicated in the switching process (4-9). C_H gene deletions that are correlated with C_H switching have been characterized in mouse plasmacytomas (4-9), hybridomas (10), tissue culture variant myeloma cell lines (11) and lymphomas (12). C_H gene deletion events have been utilized to determine the overall organization of the C_H gene cluster which has subsequently been confirmed by molecular cloning (i.e. μ-δ-γ_3-γ_1-γ_{2b}-γ_{2a}-ϵ-α) (13). C_H gene deletions associated with switch-recombination have been proposed to occur by either intrachromosomal deletions (4,9) or by sister chromatid exchanges (14).

Two models have been proposed for the molecular mechanism(s) of C_H switch-recombination (15,16). The first suggests that C_H class specific

0305-1048/82/1019-5993$ 2.00/0

DNA recombinases recognize intrinsically different repetitive DNA sequences localized within intervening DNA sequences 5' of each C_H gene (S regions) (15). The second model does not preclude the basic tenants of the first but instead proposes that two short sequences (i.e. GAGCT and GGGGT) common to all S regions mediate C_H switching by homologous DNA recombination (16). The second model is supported by the fact that a uniquely large GAGCTG repeat within S_μ and a significant portion of S_α are both conserved in mice and humans (17-19). In addition to these putative switch-recombinase recognition sequences, we have recently demonstrated that analogues of a YAGGTTG prototype sequence are found immediately 5' of the recombination sites of functionally rearranged C_H genes and are also repeated in S regions (20,21).

In this report, we present the nucleotide sequence of the $S_{\gamma 3}$ region and describe its hybridization properties with other S regions. In consideration of these results and other published cellular data on successive C_H gene switching (11,22-25), we present a model suggesting that under the appropriate circumstances the $S_{\gamma 3}$ region may allow for successive C_H gene switches.

MATERIALS AND METHODS

Molecular cloning and Southern blot hybridizations (26) were all performed as described in previous publications (20,27). The $S_{\gamma 3}$ region was subcloned into pBR322 and sequenced according to Maxam and Gilbert (28).

RESULTS

We have recently obtained a γ_3 genomic clone by screening a Balb/c sperm library with a $C_{\gamma 2b}$ cDNA probe (20). A restriction map of this clone, SL51, was derived as shown in Fig. 1. SL51 was shown to contain the $C_{\gamma 3}$ gene by comparison to other published data (29) and by cross-hybridization with a $C_{\gamma 2a}$ cDNA clone (30). To determine if SL51 contained $S_{\gamma 3}$ sequences, Southern hybridization analyses (26) of restriction enzyme digests of SL51 were performed using a variety of heavy chain switch region DNA probes. As shown in Figures 2C (lane 4) and 3A (lane 2 and 2'), strong hybridization of a 0.8 kbp RI-HindIII fragment of SL51 to both our $S_{\gamma 2b}$ and S_μ probes (PBR1.4 and pM2-20 respectively in Fig. 1) indicates that the $S_{\gamma 3}$ region lies about 1.9 kbp 5' of the γ_3 coding region (see SL51 clone map in Fig. 1). This result

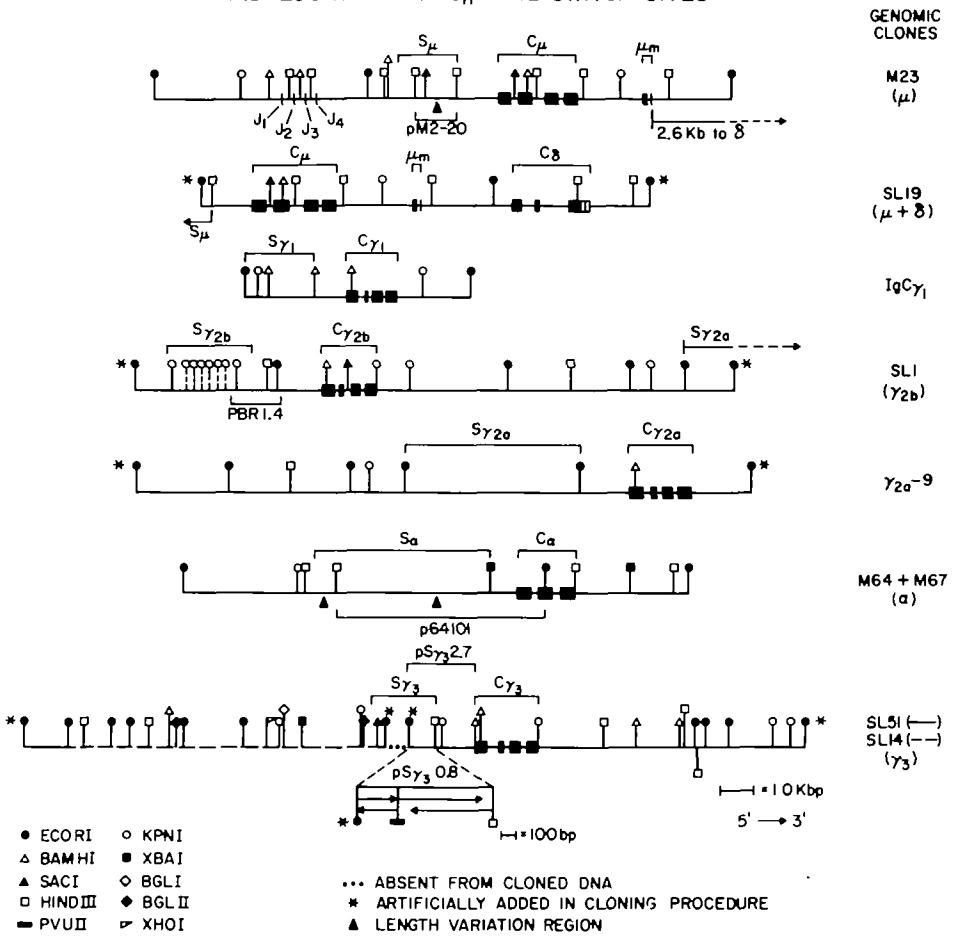
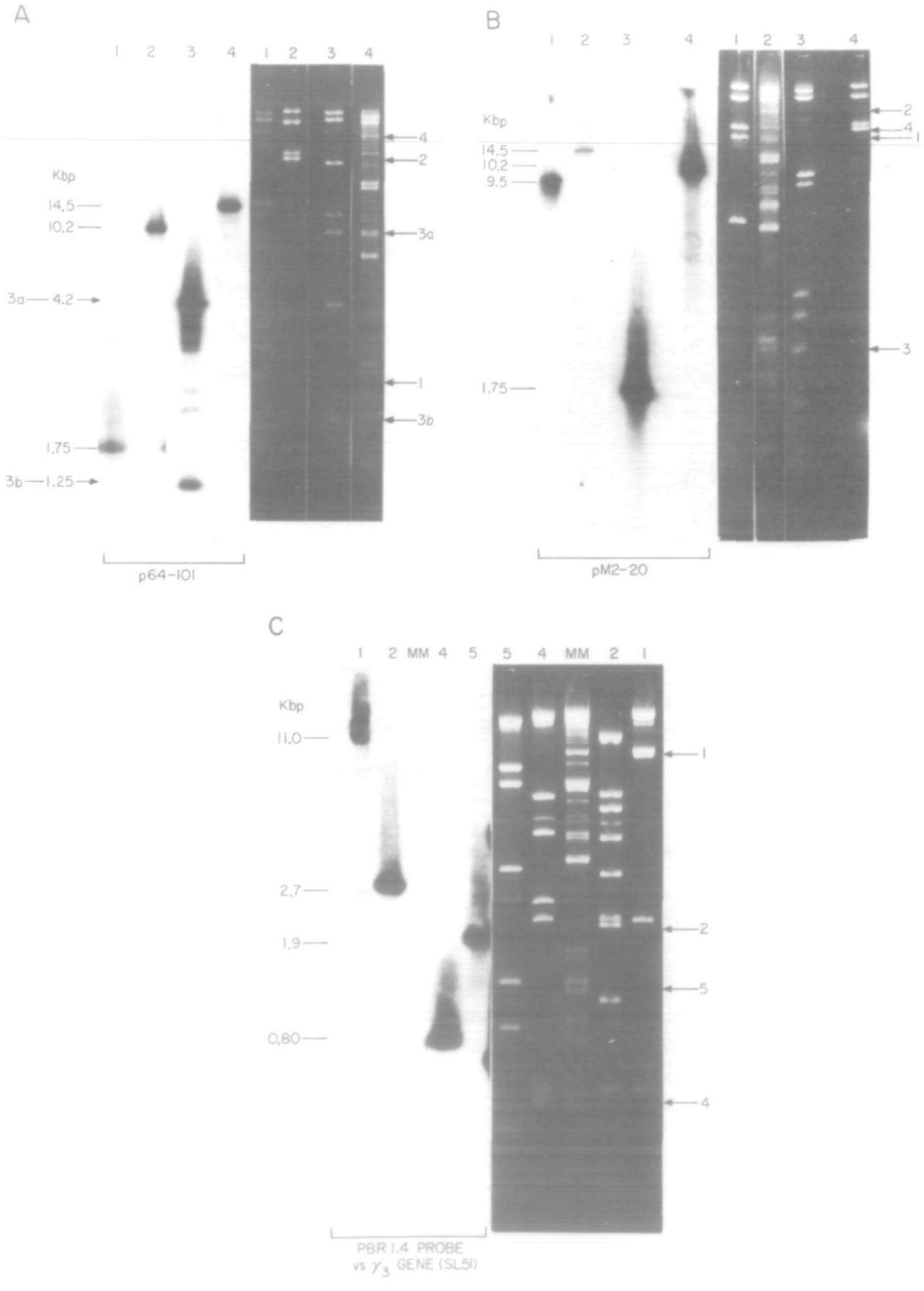


Figure 1.
Restriction maps of seven heavy chain constant region genomic clones and their 5' and 3' flanking sequence. Plasmid subclones of C_H switch regions are indicated above and below the restriction maps. The DNA sequencing strategies for $S_{\gamma 3}$ are shown below the $\gamma 3$ genomic clones SL51 + SL14. M23 (27), SL19δ (33, this paper) $IgC_{\gamma 1}$ (36), $SL1_{\gamma 2b}$ (20), $\gamma 2a$-9 (31), M64 + M67 (27), SL51 + SL14 (20, this paper).

would appear to be in good agreement with other known switch regions which are also shown in Figure 1.

While determining the location of the $S_{\gamma 3}$ region in SL51, we observed that our S_μ probe exhibited more homology to $S_{\gamma 3}$ than to our



$S_{\gamma 2b}$ containing genomic clones, SL59 and SL1 (20). To facilitate our analysis of $S_{\gamma 3}$, a 2.7 kbp RI-BamHI fragment containing the $S_{\gamma 3}$ region and 1.9 kb of 3' flanking sequences was prepared for a hybridization probe (see $pS_{\gamma 3}$ 2.7 in Fig. 1). Using an S_μ probe (pM2-20), Southern blot hybridizations were performed with the purified 2.7 kbp $S_{\gamma 3}$ containing restriction fragment, the original γ_3 genomic clone (SL51), a γ_{2b} genomic clone (SL1) that contains the entire $S_{\gamma 2b}$ region and the 5' portion of $S_{\gamma 2a}$ (20) and a restriction fragment spanning the entire $S_{\gamma 2b}$ region. The results of this experiment are shown in Figure 3A and the clones used are depicted in Figure 1. To allow for a more thorough comparison of the quantitative difference in S region homologies, 1X and 10X exposure times of this experiment are shown in Figure 3A. We have also investigated the homology of the entire $S_{\gamma 2a}$ region to S_μ by hybridizing an S_μ probe to a genomic clone containing the complete γ_{2b}-γ_{2a} intragenic region (γ_{2a}-9 in Figure 1) (data not shown). These results clearly demonstrate that S_μ possesses considerably more $S_{\gamma 3}$ homology than $S_{\gamma 2b}$ or $S_{\gamma 2a}$ homology. Cross-hybridization experiments between S_μ and S_α indicate a high degree of homology (see Figure 2A) which appears comparable to the level of S_μ-$S_{\gamma 3}$ homology.

The relative homology of $S_{\gamma 3}$ with other S regions was determined with an $S_{\gamma 3}$ probe ($S_{\gamma 3}$ 2.7 in Fig. 1). As shown in Figure 3B (lanes

Figure 2.

A. Cross-hybridization of S_α and S_μ sequences with a complete S_α probe ($p\alpha 64$-101). Track #1: HindIII digestion of μ clone M2, Track #2: EcoRI digestion of μ clone M2, Track #3: HindIII digestion of α clone M64 and Track #4: Molecular weight marker mix containing a 14.5 kb EcoRI fragment of a J558 rearranged α gene (37). Weakly hybridizing bands in Track #3 are due to deleted forms of the S_α region (32).

B. Cross-hybridization of S_μ and S_α sequences with an S_μ probe (pM2-20). Track #1: EcoRI digestion of α clone M64, Track #2: Molecular weight marker mix, Track #3: HindIII digestion of μ clone M2 and Track #4: EcoRI digestion of μ clone M2.

C. Cross-hybridizations of $S_{\gamma 2b}$ and $S_{\gamma 3}$ sequences with an $S_{\gamma 2b}$ probe (pBR1.4). Track #1: EcoRI digestion of γ_3 clone SL51, Track #2: EcoRI+BamHI double digestion of SL51, Track #3: Molecular weight marker mix, Track #4: EcoRI+HindIII double digestion of SL51 and Track #5: KpnI digestion of SL51.

All experiments were performed on 0.8% agarose gels which were transferred to nitrocellulose filters and hybridized as previously described (20,27). Ethidium stained gels are shown adjacent to the appropriate autoradiographs for comparison. Hybridizing bands are identified to the right of the ethidium pictures by their gel track numbers. Equivalent weights of DNA samples (following corrections for sequence length) were applied to all tracks.

3,4,6-8), the $S_{\gamma 3}$ region possesses extensive homology with $S_{\gamma 1}$, $S_{\gamma 2a}$ and all the repeating units that define $S_{\gamma 2b}$. A HindIII digestion of a 10.2 kb RI fragment containing the C_μ gene and neighboring sequences (see clone M2 in Fig. 1) contains only one $S_{\gamma 3}$ homologous band corresponding to the S_μ region (lanes 10 and 10' of Fig. 3B). Therefore, the $S_{\gamma 3}$ 2.7 probe does not possess detectable sequence homology to DNA sequences 3' of C_μ which span the μ-δ intragenic region (33) (see Fig. 1 for restriction maps). S_μ and S_α DNA probes also fail to hybridize to restriction fragments corresponding to the μ-δ intragenic region (see Fig. 2A (13). These findings would collectively argue that $S_{\gamma 3}$ uniquely possesses a high density of both S_μ and S_γ like sequences while DNA sequences 5' of C_δ possess no detectable S region homology. A summary of these results is present in Figure 4.

To further investigate the novel features of $S_{\gamma 3}$, we determined the complete nucleotide sequence of the $S_{\gamma 3}$ region in our $S_{\gamma 3}$ 2.7 probe. An 800 bp RI-H_3 fragment that contains $S_{\gamma 3}$ was subcloned into pBR322, and DNA sequencing strategies are shown in Figure 1. The complete sequence

Figure 3

A. Comparative cross-hybridization of $S_{\gamma 3}$, $S_{\gamma 2b}$ and $S_{\gamma 2a}$ sequences with an S_μ probe (pM2-20). Track #1: RI-BamHI insert of $pS_{\gamma 3}$ 2.7, Track #2: RI + HindIII double digestion of $pS_{\gamma 3}$ 2.7 insert, Track #3: RI + BamHI double digestion of SL51, Track #4: molecular weight marker mix, Track #5: EcoRI digestion of clone SL59 $_{\gamma 2b}$, Track #6: EcoRI digestion of clone SL1 $_{\gamma 2b}$, Track #7: KpnI digestion of clone SL1, Track #8: 3.9 kb $S_{\gamma 2b}$ restriction fragment of clone SL1, Track #9: KpnI digestion of Track #8 DNA.

B. Comparative cross-hybridization of $S_{\gamma 3}$, $S_{\gamma 1}$, $S_{\gamma 2b}$, $S_{\gamma 2a}$, S_α and S_μ with an $S_{\gamma 3}$ probe ($pS_{\gamma 3}$ 2.7). Track #1 and #2: pS 2.7 insert, Track #3: Ig $C_{\gamma 1}$ insert, Track #4: KpnI digestion of clone SL1, Track #5: Molecular weight marker mix, Track #6: 3.9 kb $S_{\gamma 2b}$ restriction fragment of clone SL1, Track #7: KpnI digestion of Track 6 DNA, Track #8: BglII digestion of Track 6 DNA, Track #9: 2.0 kb EcoRI-SacI DNA fragment from clone SL1 $_{\gamma 2b}$ (containing 1.4 kb of $C_{\gamma 2b}$ 5' flanking DNA, C_{H1} and Hinge sequences of $C_{\gamma 2b}$ as shown in Fig. 1), Track #10: pM2-20 insert, Track #11: SacI digestion of the pM2-20 insert, Track #12: HindIII digestion of $_\mu$ clone M2.

Similar quantities of DNA fragments with appropriate length corrections are applied to all tracks. Tracks 1-9' in Panel A and Tracks 1-12' in Panel B represent increased exposures obtained with intensifier screens. Ethidium pictures of the appropriate agarose gels are shown for comparison. Hybridizing bands corresponding to various C_H switch regions (see Fig. 1 for restriction maps and switch region locations) are indiated to the right of each autoradiograph and ethidium picture with their appropriate track numbers. A weak band in track 10' indicated by an asterisk (*) represents an S_μ partial digestion product. An S_μ partial digestion band is also present in lane 12'.

COMPARATIVE DNA SEQUENCE HOMOLOGIES
OF MOUSE C_H GENE SWITCH REGIONS

SWITCH REGION RESTRICTION FRAGMENTS \ HYBRIDIZATION PROBES	S_μ	5'C8	$S\gamma_3$	$S\gamma_1$	$S\gamma_{2b}$	$S\gamma_{2a}$	$S\alpha$
S_μ	++	ND	+	ND	+−	ND	+
5'C8	−	++	−	ND	−	ND	−
$S\gamma_3$	+	ND	++	ND	+	ND	+
$S\gamma_1$	+−	ND	+	++	+	ND	−
$S\gamma_{2b}$	+−	ND	+	ND	++	ND	−
$S\gamma_{2a}$	+−	ND	+	ND	+	++	−
$S\alpha$	+	ND	+	−	−	ND	++

++ = IDENTICAL SEQUENCES
+ = SUBSTANTIAL SEQUENCE HOMOLOGY
+− = LOW BUT DETECTABLE SEQUENCE HOMOLOGY
− = NEGLIGIBLE OR NO SEQUENCE HOMOLOGY
ND = NOT DETERMINED

Figure 4.
A qualitative summary of the data presented in Figures 2 and 3.

of the insert of $pS_{\gamma 3}$ 0.8 is presented in Figure 5. The γ_3 gene switch region is composed of a repeating unit of about 49 bp. This is analogous to other γ switch regions where 49 bp repeats have also been described (34). The $S_{\gamma 3}$ repeating units would appear to be more highly conserved that the repeats within other S_γ regions (34). A prevalent sequence for the $S_{\gamma 3}$ repeating unit was deduced from the nucleotide sequence. A comparison of the $S_{\gamma 3}$ prevalent sequence to the consensus sequences of other heavy chain switch regions is presented in Figure 6. The homologies exhibited between $S_{\gamma 3}$ and the other S_γ regions detected by hybridization experiments in Figures 2C and 3B are easily explained. All S_γ regions exhibit a relatively high degree of sequence homology. In addition, the degree of sequence homology shared by the four S_γ regions would appear to reflect the chromosome order of the γ genes

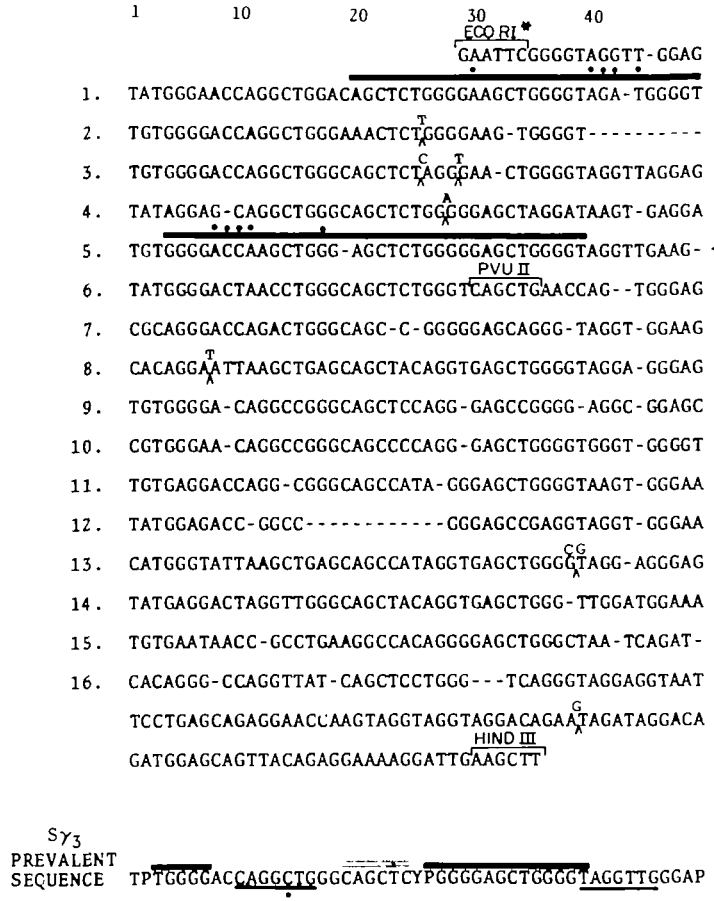


Figure 5

Nucleotide sequence of 883 nucleotides of the EcoRI-HindIII insert of $pS_{\gamma 3}$ 0.8. DNA sequence analysis was performed as described (20, 28, 36). DNA sequence is displayed in 49 bp repeating units. EcoRI and HindIII sites were labeled with ($-^{32}P$) dXTPs (> 3,000 Cimmol) and Klenow polymerase essentially as described (38) and the solitary internal PvuII site was labeled with (γ-^{32}P) ATP and polynucleotide kinase (28). GAGCT and GGGGT sequences common to the S_μ repeat are indicated by overhead solid bars. CAGCTC sequences are indicated by overhead stippled bars. YAGGTTG analogues (21) are underlined. A YAGGTTG like sequence is defined as such if at least four matched, contiguous nucleotides are present (21). Mismatched nucleotides are marked by solid dots. Two $S_{\gamma 3}$ 49 mer repeat units which closely resemble an S_μ like tandem repeat are denoted by arrows to the right of the sequence.

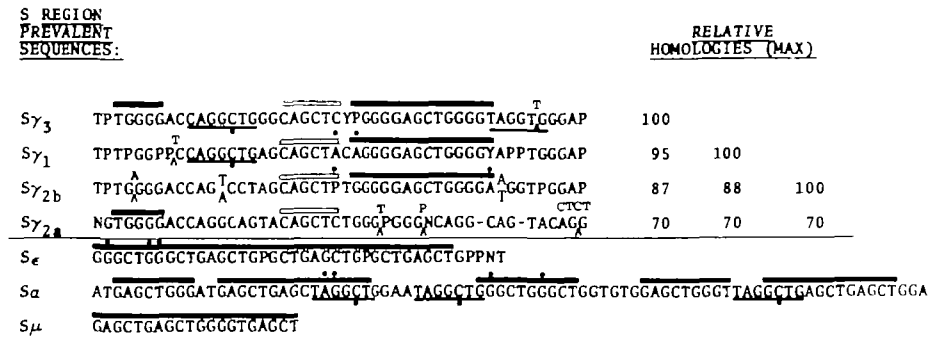


Figure 6

Comparisons of structural features of prevalent repeat sequences of $S_{\gamma 3}$ (this paper), $S_{\gamma 1}$ (34), $S_{\gamma 2b}$ (34), $S_{\gamma 2a}$ (35), S_{α} (14,15), S_{μ} (16) and S_{ϵ} (35). S_{γ} sequence homologies were determined on the basis of a 48 mer consensus repeat length and are indicated to the right of each S_{γ} prevalent sequence. Gaps and insertions were introduced to yield the maximum length of homologous DNA sequences. S_{γ} homology to S_{ϵ}, S_{α} and S_{μ} were not determined due to their radically different repeat lengths.

(i.e. $S_{\gamma 3}>S_{\gamma 1}>S_{\gamma 2b}>S_{\gamma 2a}$). However, the high degree of sequence homology between the γ switch regions does not help to explain why $S_{\gamma 3}$ uniquely displays strong homology to S_{μ} while the other γ switch regions only weakly hybridize to S_{μ}. The $S_{\gamma 3}$ sequence contains 35% more of the S_{μ} repetitive sequences, GAGCT and GGGGT, than an analogous length of $S_{\gamma 2b}$ (34), and this is reflected to some degree in the $S_{\gamma 3}$ prevalent sequence (see Fig. 6). The reason for the high S_{μ}-$S_{\gamma 3}$ homology exhibited in the hybridization experiments is also evident from the fact that two of the $S_{\gamma 3}$ 49 bp units contain an internal tandem repeat which closely resembles the sequence organization of the S_{μ} tandem repeat (16). $S_{\gamma 3}$ also possesses the short sequence CAGCTC in most of its repeating units. This sequence is the complement of GAGCTG, the highly repeated 6 mer found within the μ switch region. These results indicate that both DNA strands of the $S_{\gamma 3}$ region have sequence homology to the S_{μ} repeats.

DISCUSSION

We have presented the nucleotide sequence of the 3' end (~900 bp) of the Balb/c $S_{\gamma 3}$ region. Using these results and data obtained by others, the entire length of $S_{\gamma 3}$ is estimated to be ~4 kbp which is

comparable to the size of $S_{\gamma 2b}$ (34). However, the 49 bp $S_{\gamma 3}$ repeat unit would appear to be poorly conserved at the extreme 5' and 3' ends of the $S_{\gamma 3}$ region (see Fig. 5) (34, this paper). The switch-recombination site of the expressed γ_3 gene of the J606 Balb/c myeloma has recently been determined and is located ~300 bp 5' of the $S_{\gamma 3}$ sequence reported here (35). The $S_{\gamma 3}$ 49 bp repeating unit near the J606 γ_3 gene recombination site is virtually identical in nucleotide sequence characteristics to the ~900 bp of $S_{\gamma 3}$ sequence presented in this report.

Hybridization and DNA sequence data presented here show that the γ_3 switch region possesses substantially more S_μ homology than observed with other S_γ regions. $S_{\gamma 3}$ contains a high density of the S_μ repetitive sequences, GAGCT and GGGGT. Examples of YAGGTTG like sequences, which have been implicated in C_H switch-recombination (20,21), are more prevalent in $S_{\gamma 3}$ than in other S_γ regions (see Fig. 6). The degree of divergence of S_μ like sequences within the different S_γ regions appears to follow the C_γ gene order (i.e. $S_{\gamma 3} < S_{\gamma 1} < S_{\gamma 2b} < S_{\gamma 2a}$) (see Fig. 6). However, S_ε and S_α possess the highest degree of S_μ homology (13,35, this paper) even though the ε and α genes follow γ_{2a} in the C_H gene order (13,31).

Implications for Successive C_γ Gene Switching

When a switch from C_μ to a C_γ gene occurs during an immune response, an initial encounter with $S_{\gamma 3}$ may help to facilitate further switching to other C_γ genes. The strong homology between S_μ and $S_{\gamma 3}$ and the high density of other switch site recognition sequences in $S_{\gamma 3}$ (i.e. YAGGTTG like sequences) could conceivably favor such a phenomenon. At this point, either the γ_3 gene would be expressed or a transient S_μ-$S_{\gamma 3}$ recombination event would allow switching to continue to downstream genes. The homologies displayed between $S_{\gamma 3}$ and the other S_γ regions which are not shared by S_μ would accommodate this subsequent event. In addition, we would also predict that C_ε and C_α switches would not require $S_{\gamma 3}$ mediation since S_ε and S_α already possesses extensive homology with S_μ (13,32,35). This model would require an initial recombination event between the S_μ GAGCT tandem repeat (16) and the homologous $S_{\gamma 3}$ region. We have recently shown that the S_μ sites employed by nine rearranged C_H genes (representing γ_1, γ_{2b}, and α isotypes) are located 3' of a YAGGTTG concensus sequence and not within the S_μ GAGCT repeat (21). GAGCT tandem repeats do not appear to directly participate in most C_H gene switches and appear to be deleted

in the initial recombinatin event (21). However, the S_μ site employed by the J606 γ_3 gene is found within S_μ tandem GAGCT sequences (35).

Myeloma and hybridoma cell lines are known to possess the potential to undergo successive C_H gene switches in vitro. The MPC-11 myeloma is capable of generating $\gamma_{2b} \rightarrow \gamma_{2a}$ switch variants (22) by a DNA rearrangement which deletes the parental cell's expressed γ_{2b} gene (11). The X-63 hybridoma cell line undergoes spontaneous successive C_H switches (i.e. $\gamma_3 \rightarrow \gamma_1 \rightarrow \gamma_{2b} \rightarrow \gamma_{2a}$) at low frequency (i.e. 10^{-6}-10^{-7} per cell per generation) which are correlated with C_H gene rearrangements (23,24). The role of a successive C_H gene switching pathway in normal B cell isotype switches remains to be determined.

Recent observations by Mongini, Paul and Metcalf on the C_H switching patterns of normal B cell clones responding to TNP ficoll (a T cell independent, Type II antigen) would be compatible with a successive C_H gene switching mechanism (25). In this study, B cell clones reacting with TNP-ficoll were prepared by the splenic-focus procedure in irradiated nude mouse recipients (25). All cultured B cell clones reacting with TNP-ficoll were found to express C_μ. However, the majority of B cell clones which also co-expressed a γ isotype other than γ_3 invariably expressed γ_3 as well. These results in combination with the unique structural features of $S_{\gamma 3}$ defined in this report would collectively argue that switches to C_γ genes may proceed through an initial $S_\mu \rightarrow S_{\gamma 3}$ event in some instances. An initial $S_\mu \rightarrow S_{\gamma 3}$ event may be preferred in certain immune responses (i.e. T cell Independent responses) but need not be an obligatory prerequisite for all $\mu \rightarrow \gamma$ switches. Interestingly, B cell clones which produce IgA and IgE antibody have a much lower proportion of cells which also produce γ_3 (P. Mongini, personal communication). This would suggest that $\mu \rightarrow \alpha$ and $\mu \rightarrow \varepsilon$ switches within B cell clones are not necessarily proceeded by an initial $S_\mu \rightarrow S_{\gamma 3}$ event. This latter finding nicely agrees with the extensive S_μ sequence homology shared by both S_ε and S_α which would presumably obviate the need for $S_{\gamma 3}$ participation in successive switching to the C_ε and C_α genes. However, successive, but not necessarily stepwise, isotope switching has been observed in B cell lines which eventually express IgA (39).

We consider the idea of $S_{\gamma 3}$ participation in successive C_H switching a working hypothesis. Results obtained with spontaneously arising class switch variants clearly indicate that B cells have the

potential to undergo successive C_H gene switches (11-24). We also believe that the excellent correlation between the results presented here and the cellular data on the TNP ficoll immune response (25) is a compelling argument for such a model. Indeed, one could also argue that C_H switching in other T cell independent immune responses may occur through $S_{\gamma 3}$ participation. An excellent way to test part of our hypothesis would be to search for footprints or vestiges of $S_{\gamma 3}$ in the switch-recombination sites of functionally rearranged γ_1, γ_{2b} or γ_{2a} genes obtained from hybridomas prepared with B lymphocytes responding to TNP-ficoll or a T cell independent mitogen like lipopolysaccharide (LPS).

ACKNOWLEDGEMENTS

We thank Meryl Diamond for technical assistance and T. Honjo for supplying us with CH4A clone γ_{2a}-9.

We are grateful to Drs. William Paul, Patricia Mongini and Linda Harris for helpful, stimulating discussions during the preparation of this manuscript. We thank Patricia Mongini, William Paul and Tasuku Honjo for supplying us with preprints of their manuscripts prior to their publication. We would also like to especially acknowledge the important suggestions of Patricia Mongini in helping us to correlate our results with her recent findings on C_H gene switches during TNP ficoll immune responses. This research was supported by NIH grants GM 26939 and AI 000416. KBM is a Research Career Development Awardee of the National Institute of Allergy and Infectious Diseases.

REFERENCES

1. Nossal, G.J.V., Warner, N. and Lewis, H. (1971) Cell. Immun. 2, 41-53.
2. Raff, M.C. (1976) Cold Spring Harb. Symp. Quant. Biol. 41, 159-162.
3. Pernis, B., Forni, L. and Luzzati, L., ibid. 175-183.
4. Honjo, T. and Kataoka, T. (1978) Proc. Natl. Acad. Sci. USA 75, 2140-2144.
5. Coleclough, C., Cooper, D. and Perry, R.P. (1980) Proc. Natl. Acad. Sci. USA 77, 1422-1426.
6. Cory, S. and Adams, J.M. (1980) Cell 19, 37-51.
7. Rabbitts, T.H., Forster, A., Dunnick, W. and Bentley, D.L. (1980) Nature 283, 351-356.
8. Cory, S., Jackson, J. and Adams, J.M. (1980) Nature 285, 450-456.
9. Yaoita, Y. and Honjo, T. (1980) Nature 286, 850-853.
10. Hurwitz, J., Coleclough, C. and Cebra, J. (1980) Cell 22, 349-359.
11. Eckhardt, L., Tilley, S., Lang, R.B., Marcu, K.B. and Birshtein, B.K. (1982) Proc. Natl. Acad. Sci. USA, 79, 3006-3010.

12. Stavnezer, J., Marcu, K.B., Sirlin, S., Alhadeff, B. and Hammerling, U. (1982) Mol. Cell. Biol., in press.
13. Shimizu, A., Takahashi, N., Yaoita, Y. and Honjo, T. (1982) Cell 28, 499-506.
14. Obata, M., Kataoka, T., Nakai, S., Yamagishi, H., Takahashi, N., Yamawaki-Kataoka, Y., Nikaido, T., Shimizu, A. and Honjo, T. (1981) Proc. Natl. Acad. Sci. USA 78, 2437-2441.
15. Davis, M.M., Kim, S.K. and Hood, L. (1980) Science 209, 1360-1365.
16. Nikaido, T., Nakai, S. and Honjo, T. (1981) Nature 292, 845-858.
17. Ravetch, J.V., Kirsch, I.R., Leder, P. (1980) Proc. Natl. Acad. Sci. USA 77, 6734-6738.
18. Takahashi, N., Nikai, S. and Honjo, T. (1980) Nuc. Acids Res. 8, 5983-5991.
19. Rabbitts, T.H., Forster, A. and Milstein, C.P. (1981) Nuc. Acids Res. 9, 4509-4524.
20. Lang, R.B., Stanton, L.W. and Marcu, K.B. (1982) Nuc. Acids Res. 10, 611-630.
21. Marcu, K.B., Lang, R.B., Stanton, L.W. and Harris, L. (1982) Nature 289, 87-89.
22. Francus, T. and Birshtein, B. (1978) Biochemistry 17, 4324-4331.
23. Radbruch, A., Liesegang, B. and Rajewsky, K. (1980) Proc. Natl. Acad. Sci. USA 77, 2909-2913.
24. Beyreuther, K., Bovens, J., Dildrop, R., Dorff, H., Geske, T., Liesegang, B., Muller, C., Neuberger, M.S., Radbruch, A., Rajewsky, K., Sablitzky, F., Schreier, P.H. and Zaiss, S. (1981). ICN-UCLA Symposium on Molecular and Cellular Biology, Vol. XX (C. Janeway, E.E. Sercarz, H. Wigzell and C.F. Fox eds.) Academic Press, New York, 229-244.
25. Mongini, P.K.A., Paul, W.E. and Metcalf, E.S. (1982) J. Exp. Med. 155, 884-902.
26. Southern, E.M. (1975) J. Mol. Biol. 98, 503-517.
27. Marcu, K.B., Banerji, J., Penncavage, N.A., Lang, R. and Arnheim, N. (1980) Cell 22, 187-196.
28. Maxam, A.M. and Gilbert, W. (1980) Meth. Enzymol. 65, 499-559.
29. Adams, J.M., Webb, E., Gerondakis, S. and Cory, S. (1980) Nuc. Acids Res. 8, 6019-6032.
30. Sikorav, J.L., Auffray, C. and Rougon, F. (1980) Nuc. Acids Res. 8, 3143-3155.
31. Shimizu, A., Takahashi, N., Yamawaki-Kataoka, Y., Nishida, Y., Kataoka, T. and Honjo, T. (1981) Nature, 289, 149-153.
32. Marcu, K.B., Arnheim, N., Banerji, J., Penncavage, N.A., Seperack, P., Lang, R., Miesfield, R., Harris, L. and Greenberg, R. (1980) Cold Spring Harb. Symp. Quant. Biol. 45, 899-911.
33. Liu, C.P., Tucker, P.W., Mushinski, J.F. and Blattner, F.R. (1980) Science 209, 1348-1353.
34. Kataoka, T., Miyata, T. and Honjo, T. (1981) Cell 23, 357-368.
35. Nikaido, T., Yamawaki-Kataoka, Y. and Honjo, T. (1982) J. Biol. Chem. 257, 7322-7329.
36. Sakano, H., Rogers, J.H., Huppi, K., Brack, C., Trannecker, A., Maki, R., Wall, R. and Tonegawa, S. (1979) Nature 277, 627-633.
37. Harris, L.J., Lang, R.B. and Marcu, K.B. (1982) Proc. Natl. Acad. Sci. USA, 79, 4175-4179.
38. Sakano, H., Huppi, K., Heinrich, G., and Tonegawa, S. (1979) Nature 280, 288-294.
39. Gearhart, P.J., Hurwitz, J.L. and Cebra, J.J. (1980) Proc. Natl. Acad. Sci. USA, 77, 5424-5428.