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Structural analysis of both products of a reciprocal translocation between c-myc and immunoglobulin loci in Burkitt lymphoma

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ABSTRACT

The balanced translocations that occur between the c-myc and immunoglobulin loci in Burkitt lymphoma provide an unusual opportunity to analyze both products of a reciprocal recombination. Accordingly, we have determined the structure of the two reciprocal products of a translocation that joins the 5' portion of the c-myc gene on chromosome 8 to the immunologulin μ switch recombination signal on chromosome 14. By determining the nucleotide sequences at the translocation crossover points of both product chromosomes, we precisely locate these points with respect to nearby genes. This determination allows us to conclude that translocation involves non-homologous recombination, is highly conservative of c-myc sequences (deleting only 16 bp at the crossover point), but deletes over 2 Kb of immunoglobulin sequences from the μ switch signal. The μ constant and c-myc genes are joined head-to-head about 3 Kb apart, while the IgH enhancer and an aberrantly rearranged D/J region are linked to sequences 5' of c-myc on the reciprocal product.

INTRODUCTION

The occurrence of reciprocal translocations between the c-myc gene and the immunoglobulin loci in Burkitt lymphoma provides a rare opportunity to analyze both the substrates and the products of a reciprocal recombination that occurs in a human cell (1-3). Such analyses are important because they reflect the mechanisms by which these translocations occur and allow us to explore the structural and functional consequences of juxtaposing genes normally located on different chromosomes. Several reciprocal translocations have been analyzed in mouse plasmacytomas where recombination generally involves the immunoglobulin α switch signal (1,4-10), a sequence thought to be involved in specific recombinational events that occur during maturation of B lymphocytes and formation of antibody genes. In human Burkitt cells, these translocations generally--but not always--involve a different recombination signal, the μ switch, a sequence that occurs near the region encoding the immunoglobulin μ heavy chain (1,11-13).

A less common class of translocation involves the c-myc gene and the λ

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light chain locus (14). One example has been exmained in particular detail and has been found to involve a highly conservative recombination event that joins a region near the λ constant region genes to a point several thousand bases downstream (3') from the c-myc gene (15). In this case no switch region signal is found on either segment involved in the translocation. Because the basis of these recombination events is still not clear, we have examined both reciprocal products of a translocation that has occurred between the c-myc gene and the immunoglobulin μ switch region in the Burkitt lymphoma BL22. In contrast to the example cited above, the reciprocal exchange is relatively conservative of c-myc sequences, but not of μ switch region in which a 2 Kb segment is lost from the products of the translocation. Furthermore, the translocation has not associated the IgH enhancer (16-18) with the c-myc gene, rather the enhancer is found on the product reciprocal to the μ constant-c-myc linkage. The segment carrying the rearranged c-myc gene remains transcriptionally active, though deregulated (19,20).

MATERIALS AND METHODS

Burkitt Lymphoma Cell Line, BL22

The Burkitt lymphoma cell line BL22 was established from a pleural effusion of a 19 year old Congolese female (14). The malignant cells were characterized by the presence of the EBV genome, a t(8;14) and a μ,κ phenotype.

Genomic DNA Preparation and Southern Blotting

DNA samples from normal human peripheral white blood cells and the Burkitt lymphoma cell line BL22 were prepared as described previously (21). Aliquots of genomic DNA were digested with appropriate restriction enzymes (New England Biolabs), electrophoresed, blotted to nitrocellulose, and hybridized to nick-translated DNA restriction fragment probes. Specific activity of probes was typically 100-300 cpm/pg. Hybridized filters were washed at 52°C in 15 mM NaCl, 1.5 mM sodium citrate and 0.1% $NaDodSO_4$.

Genomic Cloning

The human germline c-myc genomic clone and the c-myc containing recombinant clone from BL22 were obtained as described previously (19). The reciprocal recombined (17 Kb EcoR1) fragment was obtained by screening approximately 2 x 10^5 recombinant phage λ CH4A (22) clones containing size purified 17 Kb EcoR1 restriction fragments from BL22 genomic DNA. The hybridization probe was a 1 Kb Cla-HindIII fragment (5' break; Fig. 1) from

the germline c-myc clone. The germline immunoglobulin heavy chain clone is described elsewhere (23).

DNA Sequencing

DNA sequencing was performed using the M13 "dideoxy" chain termination method (24) essentially as described in the Bethesda Research Laboratories sequencing manual (1981).

RESULTS

Identification and Cloning of c-myc Reciprocal Translocation Fragments

The two products of the reciprocal translocation that occurred in the Burkitt line BL22 were detected by making use of mapping data which indicated that the translocation breakpoint occurred about 1 Kb 5' to the c-myc promoter (19). A probe was made from a normal c-myc fragment located immediately 5' to the BL22 recombination site (5' break, Fig. 1). When this probe is hybridized to EcoR1-digested BL22 and control DNA's, it detects an unrearranged 12.5 Kb c-myc DNA EcoRI fragment in the control sample and two fragments in BL22 genomic DNA. One of the BL22 fragments corresponds to the unrearranged 12.5 Kb EcoRI fragment; the other, to a rearranged 17 Kb EcoRI restriction fragment. When a segment of the c-myc gene itself is used as a probe (Fig. 1), the unrearranged c-myc gene is again detected in both DNA samples, but a rearranged fragment 9 Kb in length, the other product of the reciprocal translocation, is detected in the tumor cells.

The 17 Kb EcoR1 fragment represents one of the two reciprocally rearranged fragments generated by the BL22 translocation. The 9 Kb EcoR1 c-myc-containing fragment represents the other (Fig. 1) and has its immunoglobulin recombination site in the middle of the μ switch signal, extending into sequences 3' to this region (16). The reciprocal of this fragment would therefore be expected to contain sequences 5' to μ switch, including the heavy chain J region cluster. A J region probe also detects a co-migrating, rearranged 17 Kb EcoR1 fragment in BL22 genomic DNA, as expected if this fragment represents the reciprocal fragment (data not shown). This 17 Kb fragment was cloned into a phage λ vector from size-purified, EcoR1 cleaved BL22 genomic DNA using the 5' break fragment (Fig. 1) as a probe. Comparative restriction mapping and blot hybridization experiments showed that this fragment contains immunoglobulin heavy chain sequences extending from J5 to the 5' border of the μ switch. In addition, this reciprocal fragment contains virtually all c-myc sequences 5' to the previously determined site of translocation (Fig. 1). The 17 Kb reciprocal fragment also

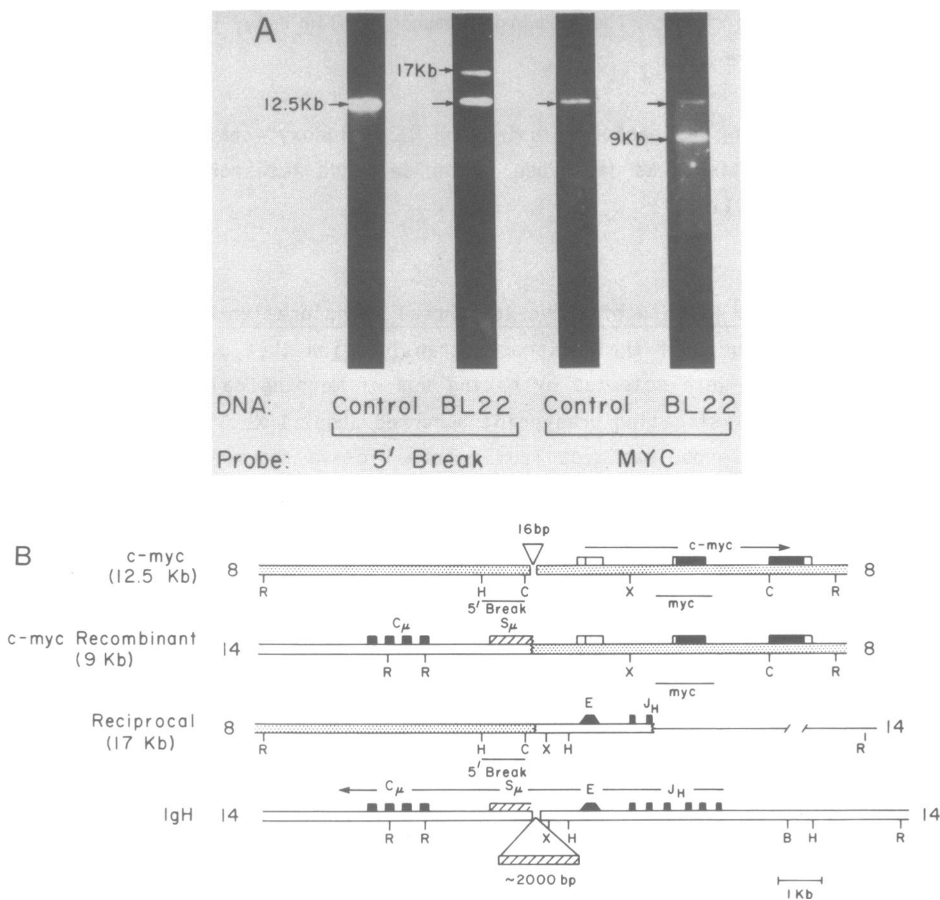

Fig. 1. Structure of germline and recombinant DNA segments around the c-myc-IgH translocation sites. A) Genomic DNA from normal human polymorphonuclear cells (control) and the Burkitt lymphoma cell line BL22 was digested with EcoR1, blotted, and hybridized with either a 5' break or c-myc probe. The map positions of these two probes on the germline c-myc fragment are designated below in part B. Both probes identify an unrearranged 12.5 Kb EcoR1 fragment in control and BL22 DNA. The myc probe detects an additional 9.0 Kb EcoR1 fragment in BL22 which is the c-myc recombinant. The 5' breakpoint probe hybridizes to the 17 Kb reciprocal recombined fragment in BL22.

B) The structure of the human germline c-myc locus and the germline IgH locus are compared to the c-myc containing recombinant and reciprocal recombined fragment. All c-myc derived DNA (chromosome 8) is represented as a stippled bar and immunoglobulin heavy chain DNA (chromosome 14) is shown as an open bar. A thin line designates rearranged DNA of uncertain origin flanking J5 on the reciprocal recombined fragment. Translated exons are filled boxes above the bars; untranslated exons are unshaded boxes. The 16 bp deleted from germline c-myc and the 2 Kb deleted from IgH by re-

combination are indicated by triangles. A hatched box indicates immunoglobulin mu switch sequences. The directions of transcription of c-myc and IgH genes are shown by arrows. The structure of the germline IgH locus is as described by Ravetch et al. (23). Abbreviations used: Cμ = μ constant region, E = immunoglobulin enhancer element, Sμ = μ switch sequences, J_H = heavy chain J segment cluster. Restriction sites: R = EcoR1, H = HindIII, C = ClaI, X = XbaI, B = BamH1.

contains approximately 8 Kb of DNA adjacent to J5 that is not homologous to either c-myc or IgH immunoglobulin J sequences (Fig. 1 and see below).

Sixteen Basepairs are Deleted from Germline c-myc at the Site of Translocation.

DNA sequences at the translocation crossover point from both the c-myc containing recombinant (9.0 Kb R1) and the reciprocal recombinant fragment (17 Kb) were compared to their unrearranged counterparts in the germline c-myc region (12.5 Kb R1) (Fig. 2). With the exception of a 16 nucleotide segment of DNA derived from the germline c-myc locus, all germline c-myc sequences are found in either the c-myc containing recombinant or its reciprocal (Fig. 2).

About Two Kilobases of μ Switch DNA are Deleted from the Translocated Heavy Chain Locus.

The c-myc containing recombinant in BL22 contains about 1 Kb of μ switch sequence adjacent to its c-myc recombination site (16). The entire germline μ switch region extends over 3 Kb (23). Restriction mapping and genomic blotting experiments comparing the germline IgH locus with the reciprocal recombined fragment showed that the reciprocal fragment contained no switch sequences (data not shown). This result indicates that at least 2 Kb of IgH μ switch sequences are deleted at the translocation site. DNA sequence comparison of the translocation site in the reciprocal fragment and the germline IgH locus precisely locates the recombination site 50 bp 5' to the μ switch region (Fig. 1 and 2), as predicted from mapping and blotting experiments.

The possibility that this 2 Kb deletion occurred as a consequence of the cloning procedure, could be ruled out by comparing the restriction fragment length of cloned and genomic DNA. BL22 genomic DNA and the reciprocal clone were digested with HindIII, blotted and probed with the 5' break probe (Figs. 1 and 3A). This probe detects a 2 Kb HindIII fragment in the cloned DNA which co-migrates with its 2 Kb rearranged HindIII counterpart in BL22 genomic DNA (Fig. 3A). This 2 Kb HindIII fragment spans the translocation crossover point in which the switch deletion has occurred

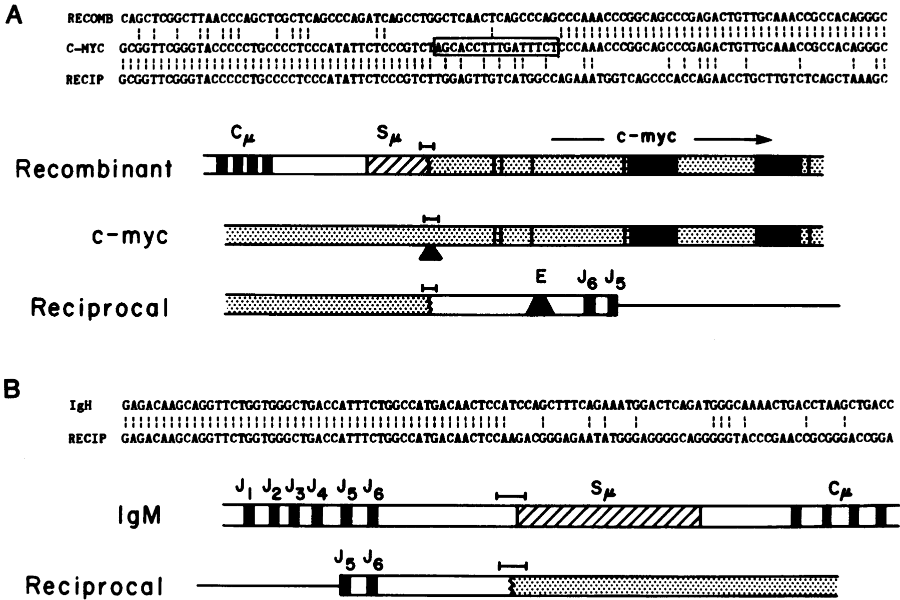

Fig. 2. Nucleotide sequences at the translocation site. A) The DNA sequences at the translocation site of the two reciprocal fragments are compared to their germline c-myc counterparts. The regions sequenced are designated by bars above the three structural representations. The 16 nucleotides deleted from germline c-myc at the recombination site are boxed. B) The nucleotide sequence of the non c-myc containing fragment (reciprocal) compared to IgH sequences bordering the μ switch. The region sequenced is designated by bars (⊢—⊣) above structural representations of these DNA segments.

(Fig. 1), since the fragments are of identical length, the deletion has occurred in the Burkitt cell. Similar comparisons of the c-myc recombinant clone and BL22 genomic restriction fragments confirmed that no major deletions occurred during cloning of this genomic fragment (data not shown).

The J Cluster on the Reciprocal Fragment has Undergone an Aberrant Immunoglobulin J-Type Recombination Event.

Restriction mapping and Southern blot comparison between a germline IgH clone (23) and the cloned reciprocal fragment from BL22 showed an abrupt break in homology between these two cloned DNAs within the J cluster (Fig. 1). In order to define precisely the location of this break in homology, DNA sequence from the reciprocal J region was determined and compared to that previously determined germline for the IgH nucleotide sequence (23). The two sequences are homologous through the 3' end of the J cluster,

but sharply diverge three nucleotides into the J5 segment (see Fig. 4). These results are consistent with a recombination event at J5.

VDJ joining is mediated by characteristic recombination signal sequences that flank germline human immunoglobulin D segments on their 5' and 3' sides (23,25). The consensus recombination signal sequence usually found on the 5' side of germline D segments is GGTTTTTGT--12 bp--CACTGTG (23,25). If the DJ-type recombination event had joined a germline D to J5, the 5' D recombination signal sequence should be found 10-30 nucleotides 5' to the J5 sequence in the reciprocal recombined fragment. No such signal sequence is found within several hundred nucleotides of the J5 segment (Fig. 4). It is clear that a normal germline D segment has not participated in a DJ recombination at J5 in the reciprocal, unless a V-D joining has also taken place.

If the recombination at J5 in the reciprocal is a VDJ joining, all recombination signal sequences associated with the joined D segment would be deleted. Instead, DNA sequence encoding a highly conserved amino acid sequence in the framework three region (amino acids 90-92) found in all known human heavy chain variable regions should be observed within 30-50 bases 5' to J5 (see Fig. 4) (26). No such amino acid sequence could be translated in any frame from DNA sequences within several hundred bases 5' to J5, ruling out a functional or aberrant V-region joining in the BL22 reciprocal recombined fragment (Fig. 4).

<u>The DNA Fragment Joined to J5 on the Reciprocal Recombinant is Homologous to a Family of D-Bearing Fragments</u>

In order to better understand the nature of the sequences rearranged 5' to J5, a probe was prepared from this region and hybridized to PvuII-cleaved genomic DNA from a normal individual and BL22. The probe hybridizes to at least four fragments in normal genomic DNA (Fig. 3B). Genomic DNA from BL22 shares one of these homologous fragments, and also contains two homologous fragments of differing sizes. The smallest homologous fragment in BL22 genomic DNA comigrates with a 748 bp PvuII-PvuII rearranged fragment containing the J5 breakpoint from the reciprocal clone (748 bp PvuII fragment is J5 break probe) (Fig. 3). In separate experiments (not shown) we find that this segment strongly crosshybridizes to a family of cloned DNA fragments known to encode multiple human D segments (U. Siebenlist, personal communication).

<u>The Nature of the Recombinational Event</u>

Comparison of the germline and translocated DNA segments from a Bur-

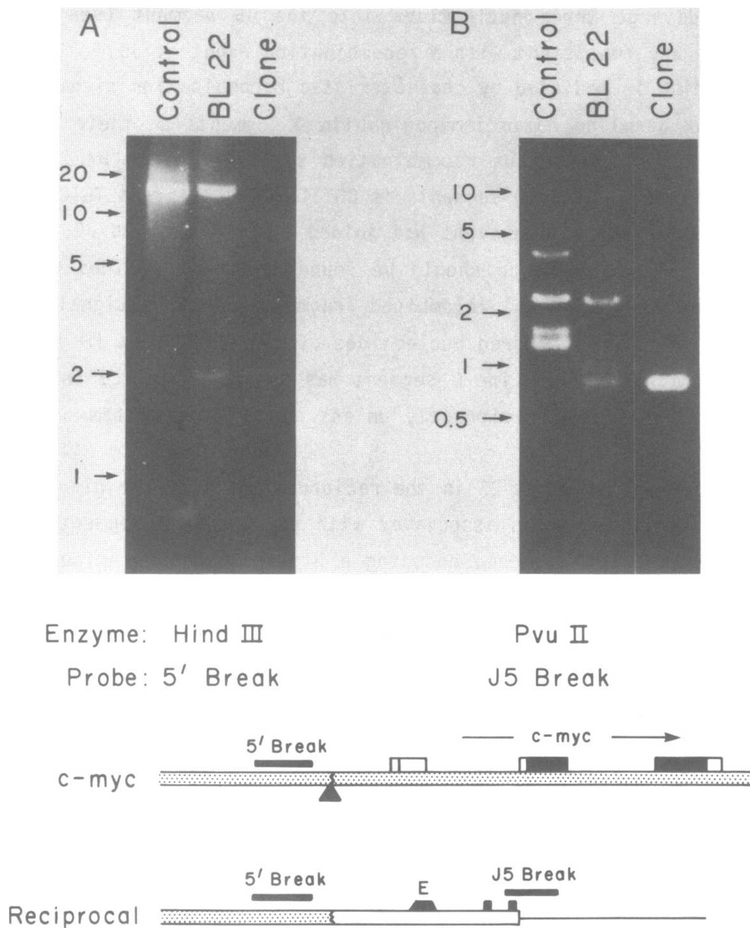

Fig. 3. Genomic representation of the reciprocally translocated junction fragment and the aberrantly rearranged J5 segment. A) Ten µg of genomic DNA from normal human polymorphonuclear cells (control) and the Burkitt lymphoma cell line BL22 are digested with HindIII and compared to 20 picograms DNA digested with the reciprocal rearranged clone in a genomic blot hybridization. The probe (5' break) hybridizes to an approximately 15 Kb unrearranged genomic fragment in control and BL22 genomic DNA. A 2 Kb HindIII fragment is rearranged by translocation in BL22 and hybridizes to the probe. The blot shows that the same 2 Kb HindIII fragment is in the reciprocal clone and comigrates with its genomic counterpart in BL22 DNA.

B) Ten µg of genomic DNA from control and Burkitt lymphoma BL22 are digested with PvuII and compared to 20 picograms of PvuII-cut DNA from the reciprocal clone by Southern blot analysis. The probe utilized in the hybridization (J5 break) is a 748 bp PvuII fragment which spans the J5 rearrangement site from the reciprocal rearranged clone. This probe hybridizes to a family of at least four genomic fragments in normal DNA. All but one of these fragments is deleted or rearranged in BL22 genomic DNA. One of the

rearranged fragments in BL22 genomic DNA comigrates with the 748 bp PvuII fragment in the cloned DNA that spans the J5 rearrangement site, clearly demonstrating that the rearrangement found in cloned BL22 DNA is not a cloning artifact.

A schematic diagram of the reciprocal rearranged fragment and germline c-myc DNA is shown below the autoradiogram. The position of the two probes used (5' Break and J5 Break) is shown in the two clones. Stippled bars denote c-myc derived sequences and open bars indicate IgH derived sequences. The BL22 translocation site is designated by a triangle on germline c-myc. The three exon structure of the c-myc gene and the direction of its transcription is indicated. The J segments and enhancer region (E) are shown on the reciprocal.

kitt lymphoma (BL22) indicates that this translocation event is highly conservative with respect to c-myc sequences in the immediate vicinity of the translocation breakpoint. This result is similar to the 8;22 variant translocation in the Burkitt lymphoma BL37, where no nucleotides are lost from c-myc sequences during recombination (8). Analysis of an analogous mouse plasmacytoma (B1-8 μ) c-myc translocation revealed loss of only 7 bp of c-myc sequences--also highly conservative (9).

Approximately two kilobases of μ switch sequences are lost from the translocation site in this Burkitt lymphoma. It is impossible to know whether this DNA was deleted before, during or after translocation, or whether the sequences were lost from either the c-myc containing recombinant or its reciprocal recombined fragment. This represents a larger deletion than has been previously observed at c-myc translocations, and may reflect the recombinational instability of this highly repeated sequence. In addition, it is important to note that the changes occurring in the immediate vicinity of the translocation breakpoint are not the only alterations detected in the structure of the c-myc gene. Detailed sequence analysis of the untranslated leader exon of BL22 c-myc gene, a region about 1.5 Kb from the crossover point described here, contains a number of point mutations and small insertions and deletions (20).

The structural basis of the recombination event is not obvious from inspection of various sequences surrounding the translocational recombination site (4-10,13,15). Clearly there is no region of evident homology in the sequences presented here. Furthermore, from a variant translocation involving the λ light chain locus, it is clear that switch region sequences are not a necessary component of the recombination site (15). What is then responsible for this recurrent non-homologous recombination event? It is still possible that some subtle feature of the primary structure of the DNA surrounding the various sites of translocation will be found (e.g. note the

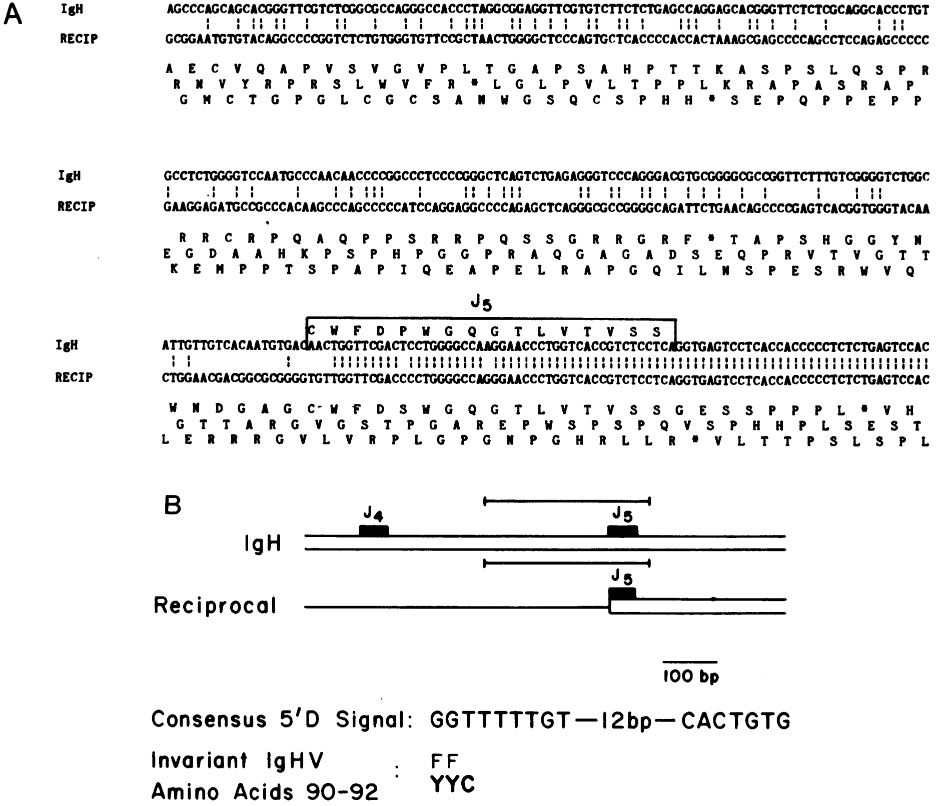

Fig. 4. Nucleotide sequence comparison of germline IgH J region and the reciprocal recombined fragment at the J recombination site. The two sequences are compared and show evidence for a J-type rearrangement three nucleotides from the 5' end of J5 in the reciprocal. The boundaries of J5 are indicated, and the J5 translation shown above its germline sequence. The DNA sequence from the reciprocal around the J5 rearrangement site is translated in three forward frames below the sequence. Asterisks designate stop codons. Neither a consensus 5' D signal nor the invariant IgH V amino acids 90-92 are found within several hundred nucleotides of the J5 rearrangement site in the reciprocal sequence. The consensus 5' D signal sequence (GGTTTTTGT-- 12 bp-- CACTGTG) and invariant amino acids 90-92 (YYC) are shown at the bottom of the figure. Phenylalanine (F) can occasionally substitute for tyrosine (Y) at amino acids 90 and 91, but the cysteine (C) at amino acid 92 is absolutely invariant. Schematic diagrams of the two DNA segments (germline IgH and reciprocal) are shown below the sequence. Bars designate the regions in which sequences are compared in the figure.

CCAAACCC found near the breakpoint in the c-myc gene (Fig. 2A) and also found in this form or as its complement near the recombination point in several Burkitt translocations (14,15) and mouse myelomas (7,9,10)). On the

other hand, since no general and consistent sequence has yet emerged, it is more useful to think of the immunoglobulin locus as one that physiologically must undergo recombination in pre-B cells and, therefore, usually exists in a chromosomal configuration suitable for recombination. Making this assumption contributes little to our understanding of why the c-myc locus should serve as a partner in this process unless we assume it is not the only fragment drawn into the immunoglobulin locus by translocation, but one of the few that leads to the dramatic transforming events that result in its detection.

The Translocated IgH Enhancer

Transcription enhancer sequences in immunoglobulin genes have been shown to markedly increase transcription in B-cells from IgH V region and other promoter sequences joined in close proximity to these enhancer elements (16,17). The human homologue to the known mouse IgH enhancer sequence (18) has been located between the J cluster and the μ switch region by DNA sequence analysis (Fig. 1). This sequence resides not with the translocated c-myc gene, but with its reciprocal in this Burkitt lymphoma. This cis-acting sequence therefore cannot be responsible for altering c-myc gene expression in this lymphoma or, in fact, in any other c-myc translocation occurring in this orientation at an immunoglobulin switch region. The translocation event has moved this enhancer near DNA sequences normally located > 1 Kb 5' to c-myc. These 5' sequences are not normally transcribed in B lymphoblastoid cells. RNA blot experiments using probes from the region 5' to c-myc show no novel transcripts in BL22 (A.R., unpublished observations). It thus appears that this enhancer does not activate new transcripts within five kilobases of the DNA fragment normally flanking the 5' portion of the c-myc gene. Furthermore, the fact that no transcripts are found corresponding to the IgH portion of the reciprocal fragment reflects the fact that the Ig V region and its promoter sequence has not yet been joined to this region.

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