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Genetics

Products of a reciprocal chromosome translocation involving the c-*myc* gene in a murine plasmacytoma

(plasmacytoma/rcp *T*(12;15) chromosome translocation/c-*myc* oncogene/γ2a switch region)

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ABSTRACT **The structures of the rearranged c-*myc* gene products derived from a rcp *T*(12;15) have been investigated in the MPC-11 (IgG2bκ) plasmacytoma. The rcp *T*(12;15) in MPC-11 is a reciprocal exchange between the c-*myc* gene on chromosome 15 and the immunoglobulin γ2a switch region ($S_{\gamma 2a}$) on chromosome 12. The c-*myc* gene is broken within a 5′-nontranslated exon, thereby separating the promoter region of the normal c-*myc* gene from its protein coding sequences. This reciprocal rearrangement results in the loss of 11 base pairs of c-*myc* sequence and 300 base pairs of $S_{\gamma 2a}$ sequence at the point of recombination. Sequences that represent the promoter region of the normal c-*myc* gene are present in the 5′-*myc* reciprocal fragment. A comparison of the nucleotide sequences at the recombination site of a number of c-*myc* rearrangements reveals a common feature that may have mechanistic importance for these translocation events.**

A number of human and murine cancers are known to possess chromosomal translocations specific to that neoplasm (1, 2). Some of these translocations have recently been found to alter the location and, in some cases, the structures of cellular oncogenes (3–11). Murine plasmacytomas possess a characteristic translocation involving chromosomes 12 and 15 at the immunoglobulin heavy-chain gene locus and at the c-*myc* gene respectively (1, 3, 4, 7–9). These translocations are apparently reciprocal exchanges that result in breakage of the c-*myc* gene (9, 10, 12). rcp *T*(**12;15**) have been shown to disrupt the c-*myc* gene within a large 5′-noncoding exon or an intron (9, 10, 12). The separation of the normal c-*myc* gene promoter sequences from the c-*myc* coding region somehow contributes to the activation of normally silent promoters within the first c-*myc* intron (9, 10). The result is a rearranged c-*myc* gene that produces truncated transcripts that are generally more abundant than the larger c-*myc* transcripts of normal cells (8, 13).

Described here are the reciprocal products of the c-*myc* gene rearrangement in MPC-11 (a γ2b κ-producing BALB/c plasmacytoma) (14). The c-*myc* gene has recombined with the switch region of the γ2a immunoglobulin gene ($S_{\gamma 2a}$) in a reciprocal fashion. The molecular cloning and nucleotide sequence of both rearrangement products have revealed the lack of complete precision in the breakage and fusion of c-*myc* with $S_{\gamma 2a}$ as judged by the loss of sequences from each region on joining. Nucleotide sequences that represent the promoter region and cap site of the normal c-*myc* mRNA are localized within the 5′ c-*myc* reciprocal fragment. A comparison of the different c-*myc* breakpoints in five plasmacytomas reveals some common structural features that may be involved in the translocation process.

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MATERIALS AND METHODS

Cell Lines and Tumors. MPC-11 is a γ2b, κ-producing mouse plasmacytoma (14). ICR 9.7.1 is a variant cell line isolated from mutagen-treated MPC-11 tissue culture cells, and it produces a M_r 75,000 heavy chain (15). PC 3741 is an IgM-producing NZB plasmacytoma. It produces a normal-sized 2.4-kilobase (kb) myc RNA (8).

Molecular Cloning and Southern Blot Hybridization. A 16-kb *Eco*RI fragment containing a rearranged c-*myc* gene (M11-myc3′) was isolated from MPC-11 DNA by preparative agarose gel electrophoresis, ligated to λ Charon 4A vector arms, *in vitro* packaged, and cloned by virtue of its hybridization to murine c-*myc* exons 2 and 3. Experiments that initially identified the c-*myc* target as $S_{\gamma 2a}$ will be presented elsewhere (unpublished data). M11-myc5′ was isolated from a gene library of ICR 9.7.1 after hybridization with a γ2b cDNA probe. The library was prepared by ligation of Charon 4A phage arms to ICR 9.7.1 genomic DNA that had been modified with *Eco*RI methylase and then partially digested under conditions that favor *Eco*RI* activity (unpublished observations).

Southern hybridizations were carried out essentially as described (16, 17).

DNA Sequence Analysis. DNA fragments were subcloned into M13 phage vectors mp8, mp9, or mp10 (18), and their sequences were determined by the dideoxy-chain-termination method (19, 20). Some sequences were determined by the reverse-priming method (21).

Nuclease S1 Mapping. A 630-base pair (bp) *Eco*RI/*Bam*HI fragment containing c-*myc* 5′ flanking and first exon sequences was 5′-end-labeled at the *Bam*HI site using polynucleotide kinase and [γ-^{32}P]ATP (22). The purified 5′-end-labeled fragment was heat denatured along with either 3 μg of PC 3741 poly(A)$^+$ RNA or yeast tRNA in 80% formamide (Fluka)/0.4 M NaCl/0.4 M Pipes, pH 6.4/1 mM EDTA at 78°C for 10 min (23). The incubation was continued at 53°C without interruption for 14 hr (23). Nuclease S1 digestion was carried out at 37°C for 30 min with 80 units of nuclease S1 (24). Digestion products were analyzed on a 6% polyacrylamide/8 M urea gel.

RESULTS

Molecular Cloning and Identification of a Reciprocal c-*myc* Rearrangement. Previous experiments suggested that MPC-11 cells contained a unique c-*myc* rearrangement that did not involve immunoglobulin heavy-chain gene switch regions (25). However, molecular cloning of the products of the c-

Abbreviations: S, switch; kb, kilobase(s); bp, base pairs.

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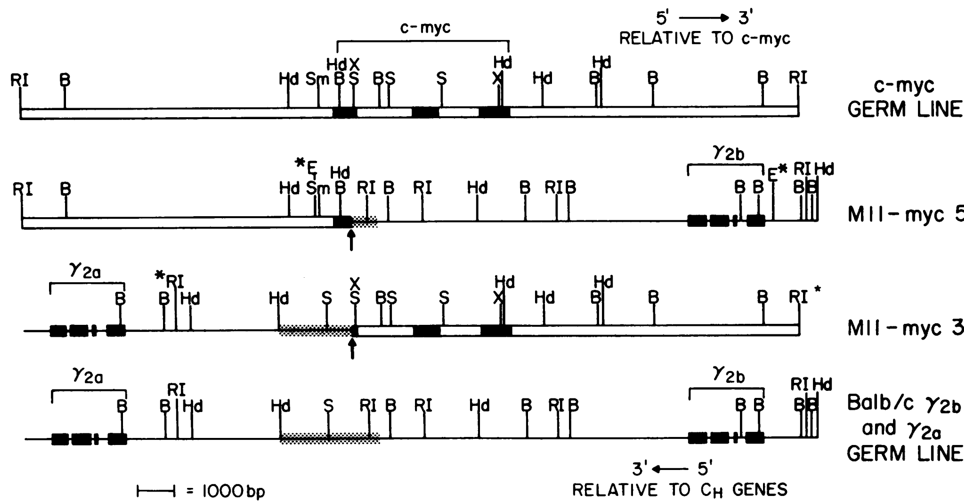

FIG. 1. Restriction maps of the c-*myc* and $S_{\gamma 2a}$ regions involved in the reciprocal chromosome exchange and the products of the recombination. M11-myc5' and 3' maps were prepared from restriction endonuclease digests of cloned DNAs and in part from genomic Southern hybridizations. Only relevant restriction sites are shown. Maps are drawn to show c-*myc* transcription from left to right requiring inverted display of the immunoglobulin regions. ——, Chromosome 12 sequences; ▭, chromosome 15 sequences; ■, coding regions; ▩, $S_{\gamma 2a}$ regions; ↑, sites of recombination; *, boundaries of clones. Restriction sites: RI, *Eco*RI; B, *Bam*HI; Hd, *Hin*dIII; S, *Sst* I; X, *Xho* I; Sm, *Sma* I; E, *Eco*RI*.

myc rearrangement in MPC-11 reveals that they were generated by a reciprocal chromosome translocation involving the c-*myc* gene on chromosome 15 and the γ2a heavy-chain-gene switch region ($S_{\gamma 2a}$) on chromosome 12 [rcp *T*(**12;15**)] (Fig. 1). As described for other c-*myc* rearrangements (3, 7–10), the 5'→3' orientation of the transcribed c-*myc* sequences (i.e., exons 2 and 3) is opposite to that of the adjacent γ2a coding region. A molecular clone corresponding to the 5' c-*myc* reciprocal fragment was isolated from an *Eco*RI* library of an MPC-11 switch variant, ICR 9.7.1. The 5' c-*myc* noncoding exon has recombined with $S_{\gamma 2a}$ sequences and resides near an intact γ2b coding region (Fig. 1). Previous work has shown that only one of the two intact γ2b genes in MPC-11 is expressed and the unexpressed gene is retained in MPC-11-derived switch variants (17, 26). We know that the γ2b gene in M11-myc5' is the unexpressed copy in MPC-11, because the expressed γ2b gene of MPC-11 has undergone deletion of CH2 and CH3 coding sequences in the formation of a hybrid γ2b/γ2a gene in ICR 9.7.1 (unpublished observations). The hybrid gene has also been isolated from the library described above and can be clearly distinguished from the intact γ2b gene of M11-myc5' (unpublished results). Genomic Southern blots carried out with *Eco*RI, *Bam*HI, and *Hin*dIII indicate that MPC-11 and ICR 9.7.1 contain the identical reciprocal c-*myc* rearrangement (see *Eco*RI results in Fig. 2).

Sequence Analysis of c-*myc* Reciprocal Clones. The nucleotide sequences at the sites of recombination in the M11-myc5' and 3' clones were determined. These sequences were compared to a c-*myc* sequence derived from a BALB/c spleen c-myc cDNA clone (10). Recombination has occurred between exon 1 of the c-*myc* gene and $S_{\gamma 2a}$ (Fig. 3). It is quite certain that both portions of the rearranged c-*myc* gene reside adjacent to $S_{\gamma 2a}$ sequences considering that (*i*) our nucleotide sequence of $S_{\gamma 2a}$ at the point of recombination in M11-myc3' indicates that it is 120 bp 3' of the *Hin*fI site shown in Fig. 3, (*ii*) the M11-myc5' target closely resembles a $S_{\gamma 2a}$ repeating unit (27) although our data do not allow precise localization of the recombination site, (*iii*) restriction endonuclease sites and sequences 5' and 3' of the precise recombination sites precisely correspond to the $S_{\gamma 2a}$ region (27), and (*iv*) probes prepared from the M11-myc5' and 3' targets and the $S_{\gamma 2a}$ region detect identical bands in genomic Southern blots (data not shown; Fig. 2).

Sequence comparisons of the recombination sites in M11-myc5' and M11-myc3' to a normal c-*myc* cDNA clone sequence indicate that on translocation 11 bp of c-*myc* are lost. By sizing restriction fragments, it is evident that a loss of about 300 bp of $S_{\gamma 2a}$ sequences has occurred (Fig. 3). The distance of the recombination site in M11-myc5' to the *Sau*3A site in $S_{\gamma 2a}$ was estimated to be 190 nucleotides. The sum of this and the 120 bp separating the *Hin*fI site in $S_{\gamma 2a}$ and the recombination site in M11-myc3' is about 300 bp less

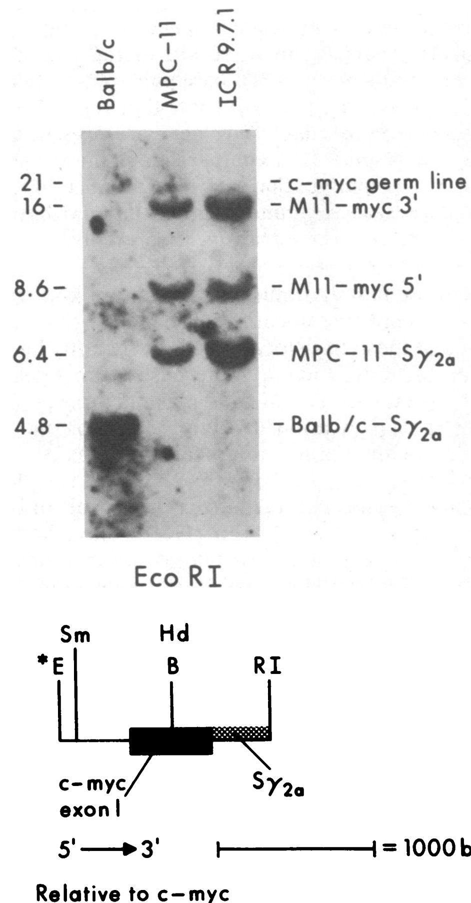

FIG. 2. M11-myc5' hybridization to genomic DNA from MPC-11, ICR 9.7.1, and BALB/c adult liver. ICR 9.7.1 is a variant of the MPC-11 cell line. Genomic DNA was digested with *Eco*RI, electrophoresed on an agarose gel (0.7%), and then blotted to nitrocellulose. The *Eco*RI/*Eco*RI* fragment diagrammed in the lower portion of the figure was subcloned in pBR325 and used as a hybridization probe in these experiments. The fragment designated MPC-11-$S_{\gamma 2a}$ maps upstream of a γ2a gene that was not involved in the c-*myc* rearrangement described here. The $S_{\gamma 2a}$ sequences flanking this gene have undergone a partial deletion with respect to BALB/c germ line (unpublished results). The other fragments detected by this probe are explained by the maps in Fig. 1. Fragment sizes (in kb) are shown.

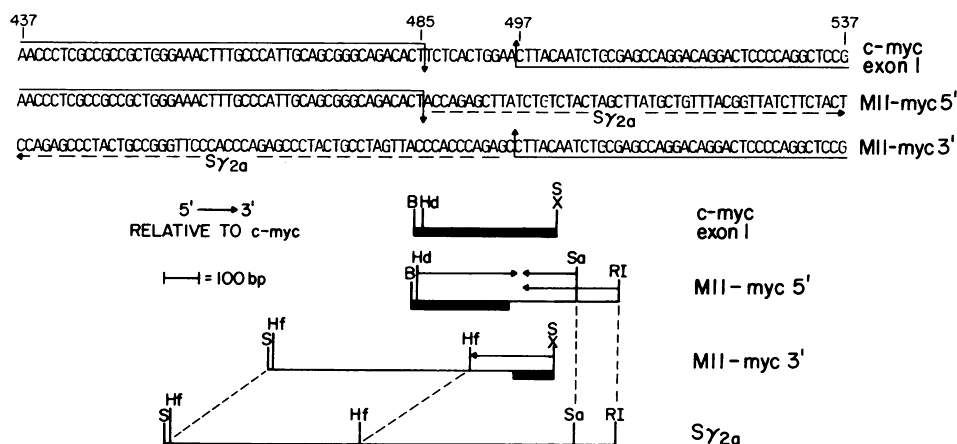

FIG. 3. Nucleotide sequences and restriction maps at the breakpoints of the substrates and products of the rcp T(12;15) in MPC-11. c-*myc* sequences within the recombination products M11-myc5' and M11-myc3' are aligned with germ-line c-*myc* sequence to indicate the breakpoints within the first exon of the c-*myc* gene. Nucleotide numbers correspond to those in Fig. 4*A*. Restriction maps are aligned to indicate loss of $S_{\gamma 2a}$ sequence on recombination. $S_{\gamma 2a}$ map is taken from other published data (27) and displayed in an inverted form. Horizontal arrows indicate the sequence analysis strategies. B, *Bam*HI; Hd, *Hin*dIII; S, *Sst* I; X, *Xho* I; Sa, *Sau*3A; Hf, *Hin*fI.

than the size of the *Hin*fI/*Sau*3A fragment (600 bp) within the BALB/c germ-line $S_{\gamma 2a}$ region (27). Therefore, a loss of about 300 bp of $S_{\gamma 2a}$ sequence has occurred between the *Hin*fI and *Sau*3A sites as a result of recombination. It is possible, however, that a nearly precise recombination may have taken place prior to or after a deletion event (see *Discussion*).

Complete Structure of c-*myc* Exon 1 and 5'-Flanking Sequences. The nucleotide sequence at the 5' end of the c-*myc* gene was determined by analyzing the appropriate region in the M11-myc5' clone. This sequence was combined with a previously determined partial c-*myc* exon 1 sequence (10), which is presented in its entirety in Fig. 4*A*. The positions of various translocation breakpoints within the first exon of c-*myc* are indicated. It has been suggested that translocation of c-*myc* results in the separation of the transcriptional control elements from the main body of the gene (9, 10). The presence of promoter-like elements within the c-*myc* sequence 5' of the breakpoints supports such a notion. The sequence T-T-T-A-T-A-T-T at positions 67–74 surrounded by several G·C base pairs resembles a "TATA" promoter sequence (28) with a potential mRNA cap site 30 nucleotides 3' at position 97 (Fig. 4*A*). A second TATA sequence is also found 155 nucleotides 3' of this one at positions 227–231 (Fig. 4*A*).

A transcription initiation site was localized 3' of the first TATA box by nuclease S1 mapping from a 5'-end-labeled *Bam*HI site located in the first exon (Fig. 5). Total poly(A)$^+$ RNA from PC 3741 (a plasmacytoma producing an increased level of a 2.4-kb normal-sized c-*myc* RNA) (8, 10) was used for this analysis. A single S1-resistant fragment of 118 nucleotides confirms that the A at position 97 in Fig. 4*A* serves as a transcription start site. The same start site was used to generate the BALB/c spleen myc RNA, which served as a template for a c-*myc* cDNA clone (Fig. 4*A*).

The first c-*myc* exon does not contain an AUG initiation codon 3' of this transcription initiation site. The presence of multiple stop codons in all three reading frames supports the idea that the first exon is noncoding (10) (Fig. 4*B*). This would confirm a previous prediction that an AUG codon followed by an open reading frame in the second c-*myc* exon serves to initiate c-*myc* translation (10). This same reading frame is interrupted by four stop codons in the first exon.

DISCUSSION

Molecular analysis of the c-*myc* translocation products in the murine plasmacytoma MPC-11 is presented. Restriction mapping, Southern hybridization, and nucleotide sequence analysis clearly show that c-*myc* on chromosome 15 has undergone a reciprocal chromosome exchange with the γ2a immunoglobulin heavy-chain gene switch region on chromosome 12. There is complete reciprocity in this exchange (i.e., the 5' and 3' ends of the broken c-*myc* gene have recombined with the $S_{\gamma 2a}$ region).

The recombination event may not be precise, because short stretches of sequence at each breakpoint are absent (11

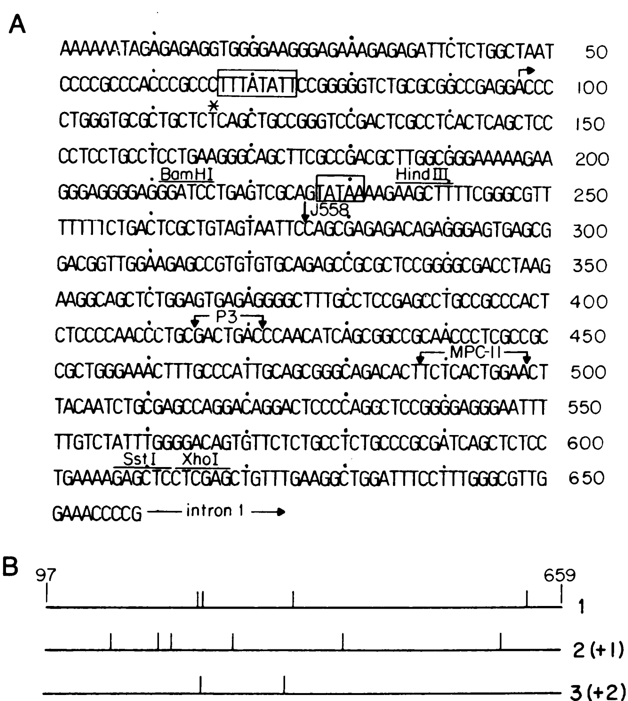

FIG. 4. Complete nucleotide sequence of c-*myc* exon 1 and 5'-flanking region. (*A*) c-*myc* exon sequences in the M11-myc5' and 3' clones were combined. These sequences are compared to the portion of c-*myc* exon 1 retained in the J558 myeloma rearranged c-*myc* gene and also to the first c-*myc* exon in a BALB/c spleen-derived c-*myc* cDNA clone. Several errors in a previously reported sequence (10) have been corrected here. We conclude from this analysis that MPC-11, J558, and BALB/c spleen cDNA-derived c-*myc* sequences are identical. The first 115 nucleotides were determined from one strand of DNA and were confirmed on resequencing. Putative promoter elements are enclosed in boxes (28). The A at position 97 represents a transcription start site as defined by nuclease S1 mapping. The first nucleotide in the aforementioned cDNA is indicated by an asterisk. The breakpoints within c-*myc* exon 1 are indicated by vertical arrows for MPC-11, J558 (10), and P3 (W. Dunnick, personal communication). The double arrows for MPC-11 and P3 indicate the 5' and 3' reciprocal breakpoints. (*B*) The stop codons in each reading frame within c-*myc* exon 1 are indicated by vertical spikes. Reading frames are indicated at the right of the figure with frame 1 corresponding to the open reading frame in exon 2 (10). The region diagrammed is from the predicted start site to the end of exon 1 (nucleotides 97–659 in *A*).

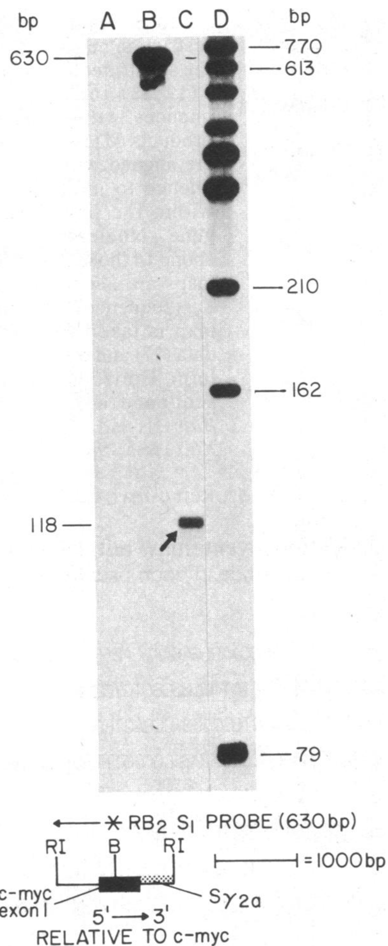

FIG. 5. Nuclease S1 mapping of normal c-*myc* RNA transcription initiation site. A 630-bp *Eco*RI/*Bam*HI fragment denoted RB_2 contains c-*myc* 5'-flanking sequences and 119 bp of exon 1. This fragment was 5'-end labeled at the *Bam*HI site as shown in the diagram below the S1 mapping gel. Hybridizations were carried out as described. Nuclease S1 digestion products and undigested controls were electrophoresed on a 6% polyacrylamide/8 M urea gel and autoradiographed. Lanes: A, 5 μg of yeast tRNA with nuclease S1 added; B, 5 μg of PC 3741 RNA without nuclease S1 added; C, 5 μg of PC 3741 RNA after addition of nuclease S1; D, 5'-end labeled *Hin*cIII digestion of φX174 DNA. A single 118-nucleotide fragment is resistant to S1 digestion after hybridization to PC 3741 RNA. The length of this protected fragment has been independently confirmed in comparison to an M13 DNA sequencing ladder.

bp of c-*myc* and ≈300 bp of $S_{\gamma 2a}$). Some of thes sequences may be lost during the exchange process. However, similar deletions may occur independently. Another γ2a gene in MPC-11, which is not involved in the c-*myc* rearrangement, has also undergone partial deletion of $S_{\gamma 2a}$ sequences (unpublished results). The loss of such small regions might also be an artifact of cloning. Immunoglobulin switch regions are notorious for their ability to delete during clonal propagation in bacteria (16). Thus, it is conceivable that a more precise exchange initially took place and the missing nucleotides were actually lost in the process of cloning these reciprocal products. In summary, the precision of this exchange cannot be fully ascertained. However, it would seem that such a reciprocal chromosome exchange can be very nearly precise with respect to the c-*myc* locus.

The cloning of M11-myc5' afforded us the ability to determine the complete nucleotide sequence of the first c-*myc* exon and its 5'-flanking region. The presence of two TATA motifs separated by 155 bp predicted the location of potential promoters for the intact c-*myc* gene. Nuclease S1 mapping

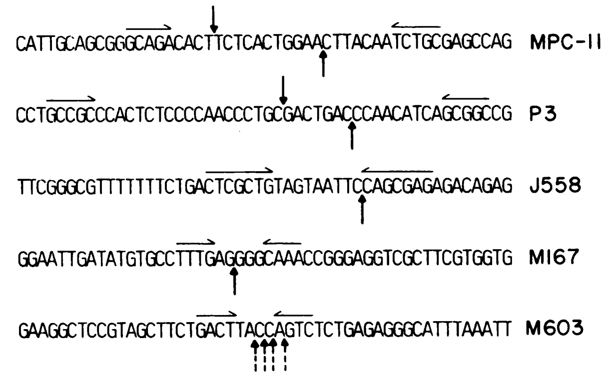

FIG. 6. Sequence comparison of c-*myc* recombination site. The nucleotide sequences of five independent c-*myc* translocation breakpoints are shown. Horizontal arrows indicate the location of short inverted repeats. Vertical arrows show the breakpoints within the indicated murine plasmacytoma. For the cases in which both reciprocal breakpoints are known, the arrow above the sequence indicates the 5' breakpoint, and the arrow below indicates the 3' breakpoint. Multiple arrows for M603 indicate ambiguity at the precise breakpoint. J558 (10), P3 (W. Dunnick, personal communication), and M167 and M603 (30).

confirmed that a transcription start site lies 25 bp 3' of the first TATA sequence. Comparable S1 experiments that would allow us to detect transcription from the second TATA indicate that this site also serves as a promoter (data not shown). The presence of multiple stop codons in every reading frame and the lack of any initiation codon within the first exon confirm the earlier suggestion that this exon is noncoding and that translation begins at the AUG found near the 5' end of exon 2 (10).

DNA sequences involved in recombination at the *myc* locus were compared to assess the molecular requirements for this translocation process. It is clear that no extensive sequence homology exists between the c-*myc* gene and the immunoglobulin heavy chain switch-region target sites on chromosome 12 as determined by the absence of hybridization between molecular clones containing these regions and from comparisons of c-*myc* and S region sequences (10, 25, 29, 30). It would therefore seem unlikely that recombination between c-*myc* and S regions is mediated by c-*myc*-associated sequences that closely resemble the tandem repeats common to S regions. The results presented here show that c-*myc* has the potential to recombine with either $S_{\gamma 2a}$ or S_{α} sequences even though $S_{\gamma 2a}$ and S_{α} repeating units possess only limited sequence homology (31, 32).

A close inspection of all known breakpoints within the c-*myc* gene reveals that other structural features may facilitate these rearrangements. As shown in Fig. 6, short inverted repeats are flanking each of five different c-*myc* breakpoints. The inverted repeats are 4–7 nucleotides long and are separated by 4–35 nucleotides. In the cases in which both products of the reciprocal exchange are known (MPC-11 and P3), both breakpoints fall between inverted repeats. These sequences could conceivably adopt some form of secondary structure recognizable by proteins involved in this type of recombination. A higher order secondary structure is implied for these rearrangements because the primary sequences of the inverted repeats are not conserved. The frequency of short palindromic sequences within the c-*myc* recombination region is no more than expected for a DNA sequence with a high G+C content (≈62% in this case). It should be pointed out that immunoglobulin switch regions are often targets for c-*myc* translocations in both murine (3, 12, 25, 29, 30, 33) and human B-cell tumors (4, 8, 9, 34) and that these regions are also known to be rich in the sequence A-G-C-T (27, 32, 35, 36). Because this sequence is palin-

dromic, switch regions may present many such structural signals for interchromosomal recombination.

Note Added in Proof. Manuscripts describing the reciprocal rearrangements of *c-myc* in P3 and in M315 have recently been published (37–39).

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