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Detection of recombinations between c-*myc* and immunoglobulin switch α in murine plasma cell tumors and preneoplastic lesions by polymerase chain reaction

(protooncogene/chromosomal translocation/gene rearrangement/plasmacytomagenesis/BALB/c mouse)

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ABSTRACT **Virtually all murine plasmacytomas carry chromosomal translocations that activate c-*myc*. The predominant (≈90%) c-*myc*-activating chromosomal translocation in pristane (2,6,10,14-tetramethylpentadecane)-induced plasmacytomas in BALB/c mice is a reciprocal translocation t(12;15) in which an immunoglobulin heavy-chain switch sequence is joined to the 5′ region of c-*myc*. The most common switch region involved is S_α. We developed a direct PCR method to screen for recombinations between c-*myc* and S_α. The critical step in establishing the method was the cloning and sequencing of the 5′ flank of C_α, a region with a reduced number of switch repeats that is much more favorable for designing specific PCR primers than the highly repetitive S_α region. In applying this PCR method, we detected translocation-specific junction fragments in transplanted (10/16, 63%) and primary (5/15, 33%) plasmacytomas. Moreover, the sensitivity of a nested version of that technique allowed us to discern rare t(12;15)s in BALB/c mice in the preneoplastic stage of plasmacytomagenesis (8/20 mice, 40%) as early as 30 days after administration of pristane. We conclude that t(12;15) is the probable primary, if not initiating, oncogenic step in plasmacytomagenesis.**

Plasmacytomas (PCTs) induced in BALB/cAnPt mice by the intraperitoneal (i.p.) injection of pristane (2,6,10,14-tetramethylpentadecane) (1) carry reciprocal chromosomal translocations t(12;15), t(6;15), or t(15;16) that are associated with the activation of the c-*myc* protooncogene (2). In the t(12;15) which occurs in 90% of the tumors the near 5′ flanking region, the first exon or part of the first intron of c-*myc* is disrupted and joined head to head with genes in the immunoglobulin heavy chain (IgH) gene complex (3–6). The most common immunoglobulin gene breakpoint is in either the α-chain switch (S_α) region (7) or the flanking region just 5′ of exon 1 of the α-chain constant region (5′-C_α).* That region (S_α/5′-C_α) is involved in 80% (28/35) of all of the PCTs with t(12;15)s that have been molecularly characterized (reviewed in ref. 8). The goal of the present study was to take advantage of these common breaksites in S_α/5′-C_α and devise a PCR method for detecting illegitimate—i.e., nonhomologous—recombinations between S_α/5′-C_α and c-*myc*. We describe here a direct PCR technique that detects 63% (10/16) of all translocation breakpoints in transplanted PCTs that are known to involve the S_α/5′-C_α region. We have applied this methodology to detect similar translocations in primary tumors and in the very early preneoplastic stages of PCT development.

MATERIALS AND METHODS

Induction, Diagnosis, and Transplantation of PCTs. Primary PCTs were induced by i.p. administration of plain pristane (2,6,10,14-tetramethylpentadecane; Aldrich) in conventionally maintained BALB/cAnPt inbred or susceptible BALB/cAnPt.DBA/2N-Idh1-Pep3 congenic mice (M.P., unpublished data). PCTs were diagnosed and transplanted as described (9). The mice were bred and maintained at Hazelton Laboratories, Rockville, MD under National Cancer Institute contract (NOI-CB-21075).

Preparation of DNA Templates for PCR. High molecular weight DNA was prepared from tissues that had been snap-frozen in liquid nitrogen or from cultured cells according to standard phenol/chloroform extraction protocols that include digestions with proteinase K (100 μg/ml, Boehringer Mannheim) and RNase A (40 μg/ml, Sigma). For rapid preparation of DNA from single cell suspensions and tissues, a lysis buffer consisting of 50 mM KCl, 10 mM Tris·HCl (pH 8.3), 2.5 mM $MgCl_2$, 0.5% Tween 20, and proteinase K at 50–300 μg/ml was employed. Cells or tissues were incubated in lysis buffer at 60°C for 4–16 h and then incubated at 90°C for 30–60 min to inactivate proteinase K. This simple in-tube protocol usually results in the release of DNA with an A_{260}/A_{280} ≈1.4 that serves effectively as a PCR template.

PCR Primers. PCR primers were initially designed by assessing available sequence data. Later, the primer analysis software OLIGO 4.1 (National Biosciences, Plymouth, MN) was employed. Altogether, 15 primers for exon 1 and intron 1 of c-*myc* and 23 for the S_α/5′-C_α region were tested. Table 1 gives the sequences for those primers found to be most reliable throughout the study. Position numbers in the S_α/5′-C_α region refer to the aligned sequence of S_α (GenBank entries MUSSREGU and MUSIGCD41) and 5′-C_α (this paper) with the first base in MUSSREGU being position 1. Oligonucleotides were prepared on a DNA synthesizer (Applied Biosystems) or purchased from Bioserve (Laurel, MD).

PCR Amplification. PCR reactions were run with reagents from Boehringer Mannheim according to their recommendations. In most cases a hot-start technique using wax pearls (Perkin–Elmer) was employed. Amounts of 10 ng to 1 μg of DNA were amplified in a final volume of 100 μl of PCR buffer consisting of 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, gelatin at 0.1 mg/ml, each primer at 0.5 μM, each dNTP at 200 μM (Boehringer Mannheim), and 2.5 units of *Taq* DNA polymerase. The PCR reaction was performed in

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Abbreviations: MOPC, mineral oil-induced plasmacytoma; TEPC, tetramethylpentadecane-induced plasmacytoma; PCT(s), plasmacytoma(s); IgH, immunoglobulin heavy chain; IgH_α, immunoglobulin heavy chain α; S_α, switch region of α chain gene; C_α, immunoglobulin α-chain constant region; 5′-C_α, 5′ flanking region of C_α; t(12;15)(s), reciprocal translocation(s) involving band F1 on chromosome 12 and band D2 on chromosome 15; t(6;15), reciprocal translocation involving band C2 on chromosome 6 and band D2 on chromosome 15; t(15;16), reciprocal translocation involving band D2 on chromosome 15 and band B1 on chromosome 16.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. L13590).

Table 1. PCR primers

Primer	Position	Sequence (5′→3′)	Length, nt
*myc*1	532–564*	TTTTATACTGCGACTCAGGATCCCTCCCCTC	31
*myc*2	1625–1659*	CCAAGTCAACGAATCGGTCACATCCCTGTCCCAAT	35
*myc*3	1677–1711*	AGGGATACCCGCGGATCCCAAGTAGGAATGTGAGG	35
α1	2109–2133†	AGCTCAGCTCAGCCTAGCCCAGCTC‡	25
α2	2363–2381†	TGTAGCCTAGCCAAGTTTA	19
α3	3170–3189†	TCACACAAAGCAAACCAGAG	20
α4	3365–3383†	GCTTCAGACCCACCACTCC	19
α5	3589–3608†	GTTTGGGCAGTGGATAGAGC	20
α6	3746–3768†	CCACAAGTTCTGGCTGTATAGAC	23
α7	113–132§	ATCAGGCAGCCGATTATCAC	20

*Base pair number in GenBank file MUSCMYC1.
†Base pair number in $S_\alpha/5'\text{-}C_\alpha$ in aligned sequence (this paper).
‡Primer has two additional perfectly complementary sites upstream in positions 1001–1025 and 922–946.
§Base pairs 113–132 in GenBank file MUSIGCD42 (complementary to a sequence 64 bp into C_α).

a PTC-100 programmable thermal controller (MJ Research, Watertown, MA) for 40 cycles at constant primer annealing and DNA melting times but steadily increasing DNA extension times. The following conditions were chosen: (*i*) initial denaturation at 95°C for 5 min; (*ii*) incubation for 10 cycles at 65°C for 15 sec, 72°C for 15 sec, and 95°C for 15 sec; (*iii*) followed by 10 cycles each at 65°C for 30 sec, 72°C for 60 sec, and 95°C for 90 sec extension time; (*iv*) final extension at 72°C for 10 min; and (*v*) indefinite holding at 4°C. The annealing temperature (T_a) needed to be optimized for each primer pair and varied between 52°C and 68°C. However, most of the routine screening experiments were done at T_a = 65°C. The second round of PCR was performed as nested PCR on a 5-μl aliquot of the first round PCR mixture. In most cases a primer pair was chosen that would be complementary as far as possible at the 5′ and 3′ ends of the first PCR product. PCR fragments in the range of 0.5–3 kb could be amplified by employing this protocol. PCR products from one- and two-round PCRs were fractionated by standard electrophoresis on 1.5–4% agarose (electrophoresis grade, BRL) gels.

Cloning and Sequencing of PCR Products. PCR products were ligated into pCRII cloning vectors and cloned in *Escherichia coli* (Invitrogen) without prior purification of the PCR product in most cases. PCR fragments were purified with Magic PCR Preps (Promega) when the initial cloning experiment was unsuccessful. Plasmid DNA was prepared from bacterial overnight cultures by using Magic Maxipreps (Promega). Dideoxy-termination DNA sequencing was performed on double-stranded DNA by using the Sequenase kit (United States Biochemical) according to the instructions of the supplier.

Cloning and Sequencing of the $5'\text{-}C_\alpha$. A rearranged 5.4-kb IgA fragment from the Abelson virus-induced PCT ABPC 60, pAB2.1, was cloned from a bacteriophage genomic library. Restriction mapping and partial sequence analysis of pAB2.1 showed that one end of the insert contained germ-line IgA sequences including the 5′ flank of C_α, whereas the other end of the clone mapped within the chromosome 15 c-*myc*/*Pvt-1* amplicon (J.S., unpublished data). Clone pAB2.1 was chosen to sequence the $5'\text{-}C_\alpha$ region, using the Sequenase method as described above.

RESULTS

PCR Amplification of c-*myc*/IgH$_\alpha$ Junctions. In preliminary studies we found that the highly repetitive tandem pentameric sequence of S_α (CTGRG) is a major obstacle for specific primer annealing and extension in that region. The S_α sequence as originally determined (10) has been recently extended (11). However, the nucleotide sequence bridging S_α and C_α—i.e., $5'\text{-}C_\alpha$—which we report here (Fig. 1), had not been previously determined. This region contains far fewer repetitive switch pentamers than S_α (see Fig. 3*C*) and therefore provides primer sites that are more suitable for initiating PCR-driven DNA synthesis through the translocation breakpoints into c-*myc*. To establish the PCR technique a panel of 16 transplanted PCTs with molecularly verified breakpoints associated with the S_α region were selected (8). When the primers shown in Table 1 were used, c-*myc*/IgH$_\alpha$ junction fragments were amplified in 10 of 16 (62%) transplanted tumors (Table 2), and the products were characterized by DNA sequencing (Table 3). Amplified products were not detected in 6 of 16 PCTs; MOPC 41, MOPC 46B, TEPC 15, TEPC 609, SAM 368, and BAL 17. The reasons for that are presently unclear. The PCR-generated junction fragments of five PCTs were compared with those previously described by cloning and sequencing of recombination joints. For the remaining five junction fragments generated by PCR previous sequence data were not available. PCR analysis confirmed the previously determined translocation breaksites for MOPC 167 (12, 13), TEPC 1165 (14), and HOPC 1 (6). Based on the better definition of the $S_\alpha/5'\text{-}C_\alpha$ region, the PCR breaksites for MOPC 315 (13) and XRPC 24 (15) in c-*myc* were located 6 and 4 bp 5′ of the sites previously described, respectively.

We extended this analysis to 15 primary PCTs and found five tumors (33%) that yielded junction fragments; the sequences of these five are given in Table 3. The t(12;15) of one tumor (Co.1) was confirmed cytogenetically (F. Wiener, unpublished result). The result of studies on primary and transplanted PCTs indicated that PCR analysis could be effective in determining when the t(12;15)s take place in

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          10        20        30        40        50        60
0001 AGCTGGCCTA AGATGGACTT AGTTGAGGTG AAGTGAGAAC TAGGCTGGAA TGGGCTTTCT
0061 GAGCTGGGCT GAAATGGCCT GAGCTGGGCT GGACTGGACT GGAATGAATT AGTCTGGGCT
0121 AGGCTGAGTT AGTCTGGGCT AGGCTAGATT AGTCTGGGCT AGGCTGAGTT AGTCTGGGCT
0181 AGGCTGAGTT AGTCTGGGCT AGGCTGAGTT AGTCTGGGCT AGGCTGAGTT AGTCTAGGCT
0241 GGACCAAATT AGGCTGGATG GGCTAAACTG AGCTGAACTA GGATGGGATG GGATGGGATG
0301 GGATGGGATG GGATGAACTG ACTGGGCTGG ACTCAGTTGA CCTTGCTCGT CTGAGCTGGT
0361 CTAGATGGTC TAGTTGGGCT GGCCAGGATA GTCAGGAACT AGGCTGGAAT TAGGCGAAAC
0421 TTGGCTTGGC TGGTTACAAT GAGCTAACAT AAATTCAGCT GGCTGAACCA AACTTGACAG
0481 TGAGCTAGCC TGGGGTGAAT TAGCATGACT GGACTTATTC ACAGTTCTAG CCTGAGCTTT
0541 GCTGGATTGT TAAACTCACT GCTGAGGTAA CTGAAAAGAC TTTGGATGAA ATGTGAACCA
0601 ACTCTTGTTT TATGGTCCAG ATTGTGGCCT CTGGTTTGCT TTGTGTGAAT GGAGCCATGC
0661 AACCTAGTAT CCTATTCTCA CACCCCCTCC TCCTTCCCTG ATTATCTGGT GGAAATTCAC
0721 ATGTACCTGT AAATGGTGCC TTCATGGCAC GGACAGAGAC ACCGCTCAGA TTGAGTCCTC
0781 TCGTCTTTGG GTCCACTGCT TGCTAAGTGG ATGGGCAGTT GCTGGAGTGG TGGGTCTGAA
0841 GCCAGAACAA GGCCAGTGTA AGGTGAAAAC ACAACAAAAT GGCCTTCTCC ACCCCACACA
0901 CAACCCCCTG CCCCTGCCAG TGTAACTTCC AAGCCAGCTC TTCCCCAACC ACTCCTACCC
0961 CGTGCTGGCT GATCTTCAGT CTCAGGTTGG CCACCCCTGC CCAGACCCAC CAGTTCTGGT
1021 CCTCCTAACC CTGGGACTGA GAACATAGCT CTATCCACTG CCCAAACAGG AGTGGGGCTT
1081 AGAAATCTGG AGCGCTAGAC TGCTCAGGGG TGTAGTCATG TTTACAAACG CACAGTATGT
1141 GCAAAGCCCT GCTAGAGTCA TTGGCTAGAT CCTTGTGAAA GACTACCTGC AGGTCATGTT
1201 CAAAGTCTAT ACAGCCAGAA CTGTTGGTCA GCTCCGACTG CGGGTACACA ATGCAGCAGC
1261 TATGTGATAC TGGGCTAGGT TCTCCTGTAT AAAGAAGAGA AAGGCATGGT CCTTTTTCCT
1321 GAAATTGTCT CGAGATGGGC AGTGTGAAGA CTACTATCTC ATGCATGTTT TATGTTCCA
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FIG. 1. DNA sequence of $5'\text{-}C_\alpha$. The consensus S_α pentamers CTGAG and CTGGG are underlined. A noteworthy region that consists of the sixfold uninterrupted repeat of the 20-bp sequence CTGGG CTAGG CTRRR TTAGT (nt 114–234) and also contains typical S_α repeats is given in italics.

Table 2. Summary of PCR results

Tissue tested	No. of mice	c-*myc*/IgH$_\alpha$ PCR junction fragments amplified		Days after pristane
		No.	%	
Transplanted PCTs*	16	10	62	NA
Primary PCTs†	13	4	31	110
Primary PCTs‡	2	1		185
Oil granulomas§	4	1		110
Oil granulomas¶	20	8	40	30

*c-*myc*-IgH$_\alpha$-positive transplanted PCTs in BALB/cAnPt mice; NA, not applicable.
†Primary PCTs in C.D2-Idh-Pep3 mice.
‡Primary PCTs in BALB/cAnPt mice.
§Oil granulomas from tumor-free C.D2-Idh-Pep3 mice.
¶Oil granulomas from tumor-free BALB/cAnPt mice.

plasmacytomagenesis. Therefore, nested PCR was applied to detect t(12;15)s in the very tissue where PCTs develop, the peritoneal oil granuloma. The intestinal mesenteric tissue of four mice 110 days after the injection of pristane was cut into five to seven sectors and then used for PCR amplification and histological examination. Three to seven plasmacytic foci per mouse were found. One mouse yielded a junction fragment (Table 2) indicating that the plasma cells in foci could have t(12;15)s. These observations were then extended to the very early oil granuloma tissue at day 30 after pristane in 20 BALB/c mice. Surprisingly, 8 mice yielded c-*myc*/IgH$_\alpha$ junction fragments (Table 2); in 6 of 8 mice the recombination joint was found in only one mesenteric sector and in 2 of 8 mice, in two sectors. Thus the majority of the sectors investigated remained negative. These results were confirmed by repeat experiments in which the pattern of positives and negatives was reproduced. The results of all PCR analyses are summarized in Table 2.

Mapping of Chromosomal Breakpoints and Structure of c-*myc*/IgH$_\alpha$ Junctions. Several c-*myc*/IgH$_\alpha$ junction fragments from each tumor were cloned, and at least one fragment per tumor was completely sequenced. The sequences adjoining the junction sites and the exact positions of the breakpoints with respect to the c-*myc* and IgH$_\alpha$ germ line are summarized in Table 3. Chromosomal breakpoints in c-*myc* (Fig. 2*B*) and IgH$_\alpha$ (Fig. 3*B*) were mapped on the basis of the sequence data. The figures show that translocation breakpoints are distributed evenly along 3 kbp of IgH$_\alpha$, whereas most of the breakpoints in c-*myc* are clustered in a 600-bp region between the end of exon 1 and the midpoint of intron 1. That region in c-*myc* could consequently be defined as a major breakpoint cluster. Rare tumors—e.g., TEPC 1165—do not break within that cluster but map in a region that is found about 500 bp 5′ of c-*myc* (17). Some general observations with respect to the chromosomal breakpoints were made (Table 3): First, most of the breaksites are clean double-strand breaks. Two tumors, TEPC 1017 and 4132, show a single nucleotide insertion exactly at the breakpoint; one tumor, 4124, has a three-nucleotide insertion. Second, the adjoining sequences are not homologous at all. Third, several base substitutions, single-base-pair insertions, or deletions were noted in the vicinity of the breakpoints. Fourth, S$_\alpha$ pentamers (CTGAG, CTGGG) and/or S$_\mu$ pentamers (CTGAG, GGGGT) were found in 13 out of 17 tumors in c-*myc* within 50 bp of the breakpoint.

DISCUSSION

Detection of c-*myc*/IgH$_\alpha$ Recombinations by PCR. The PCR method described in this paper has the potential to detect all translocation breakpoints in c-*myc* that occur in the region of the gene indicated in Fig. 2*A* as the 5′ and 3′ limit of breaksites. In contrast, the method is limited to segments of the IgH locus in several ways: (*i*) Only breaksites in IgH$_\alpha$ can be identified, although other regions of the IgH gene cluster, most notably S$_\mu$, S$_{\gamma 2a}$, and S$_{\gamma 2b}$, are known to be utilized in some PCTs (Fig. 3*A*). (*ii*) Within the entire IgH$_\alpha$ locus only breakpoints in S$_\alpha$/5′-C$_\alpha$ can be detected. This restriction potentially misses other sites in IgH$_\alpha$ such as in C$_\alpha$ and 5′ of S$_\alpha$. (*iii*) The repetitive sequence of the S$_\alpha$/5′-C$_\alpha$ region (Fig. 3*C*) limits the design of specific oligonucleotides to the 5′-C$_\alpha$

Table 3. Sequences adjoining chromosomal breakpoints on chromosome 12+

Tumor	S$_\alpha$/5′-C$_\alpha$	Tx	c-*myc*	Breaksite position*		PCR primers†	
				S$_\alpha$/5′-C$_\alpha$	c-*myc*	c-*myc*	IgH$_\alpha$
MOPC 167‡	atcccaActtagttt	-	gggTcaaaccgggag	2252	1284	3	1
MOPC 173‡	ctcagctcagcctag§	-	cctgtgTttttgaca	930	1074	2, 3	1, 4
MOPC 315‡	cccagcctattctag	-	tttgaagcggggttc	1044	1191	3	1, 5
MOPC 511‡	cagccttgttcagcc¶	-	ctacattaattgata	2415	1258	3	3, 4, 5, 7
TEPC 601‡	agcctaacccaA-tc	-	tcctttgggcgttgg	1568	967	2	2, 4, 7
TEPC 1017‡	cttaTcaagcagtgg	T	ttctgacttaccagt	3334	1393	2, 3	4, 5, 7
TEPC 1165‡	gtcaactgagtccag¶	-	ctgggctgcggggct	2868	230	1	3, 4, 5, 7
XRPC 24‡	gcttcagacccacca¶	-	cagctctcctgaaaa	3369	922	2, 3	5, 6, 7
HOPC 1‡	accagcccagcccag¶	-	ctgtgttctttctgc	1711	1016	2, 3	1, 3
HOPC 8‡	actgagctgagctca¶	-	gggctgacgCtgacc	674	1219	2, 3	3, 4, 7
4122‖	cccagcccagcctat	-	cagctctcctgaaaa	1706	922	3	4
4124‖	tccattgtagcctag	AGC	ccgccaatccccggc	2372	1476	2	3
4128‖	aaggaggaggggtg§	-	tgtcaagatgacaga	3222	1147	2, 3	4, 7
4132‖	cagccagcccttcca	C	ctctgagagggcatt	2294	1408	3	3
Co.1‖	ggaacataaaacatg	-	aggctggatttcctt	3905	957	3	7

Tx indicates translocation breakpoint; junction fragments have been generated by any combination of primers indicated in the last two columns. Sequences are given 5′→3′. Note that because of the head-to-head character of t(12;15) S$_\alpha$/5′-C$_\alpha$ sequences are reversed with respect to transcriptional orientation. Lowercase letters denote germ-line sequences, uppercase letters specify base substitutions or insertions, and a hyphen indicates a deletion.
*Lists positions of breaksites in S$_\alpha$/5′-C$_\alpha$ and in c-*myc* (GenBank entry MUSCMYC1).
†For PCR primers used for c-*myc* see Table 1 and Fig. 2*A*; for PCR primers used for S$_\alpha$/5′-C$_\alpha$ see Table 1 and Fig. 3*B*.
‡Transplanted PCT.
§Three breaksites are possible, and the most 5′ one with respect to c-*myc* is given.
¶Two breaksites are possible and the most 5′ one with respect to c-*myc* is given.
‖Primary PCT.

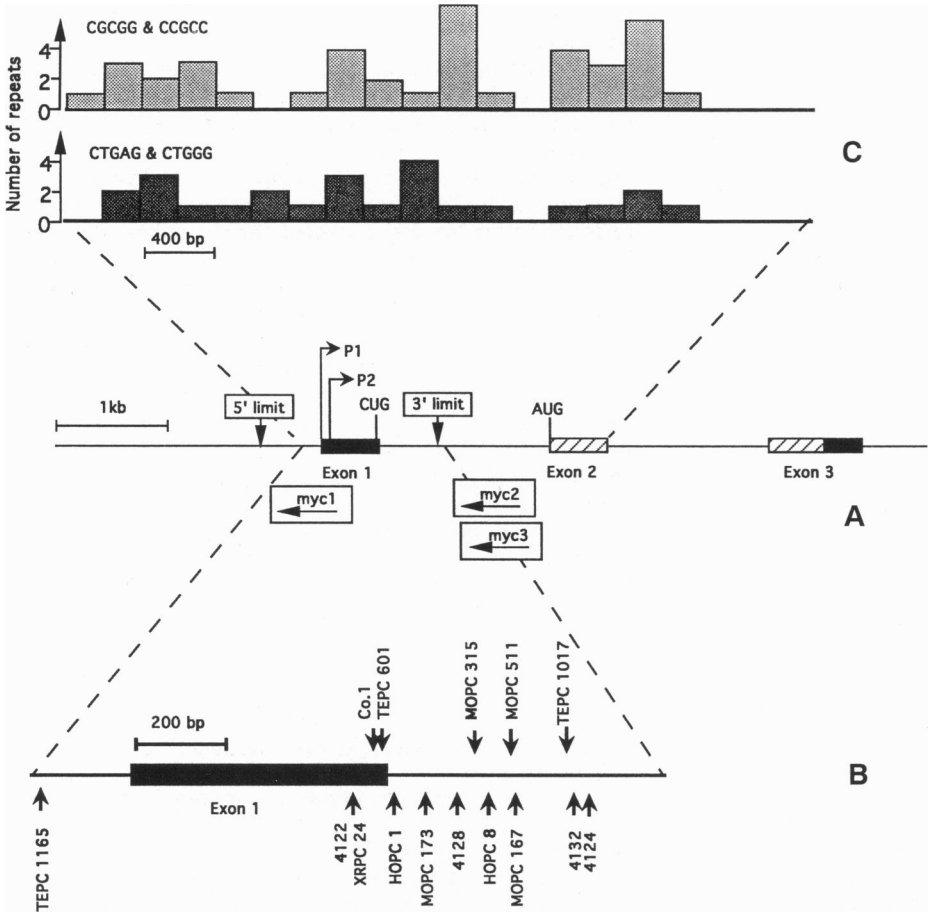

FIG. 2. (*A*) Diagram of the c-*myc* gene. The three exons of the gene are illustrated by rectangular boxes with hatched regions and filled regions representing coding and noncoding sequences, respectively. The two main promoters of the gene, P1 and P2, are found in the first exon. The c-*myc* gene codes for two distinct proteins whose initiation codons CUG and AUG are given. Translocation breakpoints occur in a region whose 5′ and 3′ limits are indicated. Complementary sites for primers myc1–3 are also illustrated (see Table 1). (*B*) Map of chromosomal breakpoints of t(12;15)s in c-*myc*. (*C*) Frequency distribution of the S_α pentamers CTGAG and CTGGG and the recombinogenic pentamers CGCGG and CCGCC in the 5′ region of c-*myc*.

region, whereas all S_α primers tested represent consensus sequences. However, using consensus primers in a highly repetitive S_α region invites mispriming and PCR artefacts. We were able to design only a single primer (α1; Table 1) for the 3-kbp S_α region (combined GenBank entries MUSSREGU and MUSIGCD41) that worked reliably. It is noteworthy, though, that so far direct PCR in immunoglobulin S regions has been restricted to the use of primers that are complementary to the nonrepetitive flanking regions (18–20). Our data, however, show that primer complementary to sequences within an S region can on some occasions be employed.

Isotype Switching and t(12;15). It is not known whether t(12;15)s are mediated by aberrant switch recombinase activities. However, the distribution of chromosomal breakpoints along S_α/5′-C_α, either within or downstream of physiologic switch recombination sites, suggests a mechanism of recombination that involves switch recombinase-mediated cuts within the S_α acceptor site region followed by deletions that move the breaksites further toward C_α (Fig. 3*B*). The hypothesis that switch recombinase is involved in t(12;15) is supported by the following facts: First, switching is not confined to the functional chromosome but occurs on both chromosomes, often at the same IgH region (21, 22). Thus switching to IgH_α on the productive allele may occur in parallel to switching to IgH_α on the nonproductive allele. It is feasible that during this process the latter allele is rendered accessible to recombination with c-*myc*. Second, the ability for such illegitimate recombination to occur may somehow be related to the imprecision of the switching process, which is known to be particularly variable for the nonproductive IgH gene locus (23). Third, c-*myc* contains numerous S_α (and S_μ) pentamers in its 5′ region (Fig. 2*C*) that had been noticed previously (6, 24). It is conceivable that these repetitive pentamers somehow facilitate recombination with S regions as suspected early on (24). However, the excess of S_α pentamers in the region of c-*myc* that is indicated in Fig. 2*C* is modest; i.e., the motifs GGGGT, CTGGG, and CTGAG occur 3–4 times more frequently than expected, and so far we have no proof for their involvement. Short direct repeats distinct from S_α pentamers may also increase the likelihood for c-*myc*/IgH_α rearrangements. In this respect, the excess of short recombinogenic motifs in the 5′ region of c-*myc* such as the tetrameric sequences GAGG (13, 25), CCCT (26), CGGC (27), and the pentameric GC stretches CGCGG/CCGCC (refs. 28 and 29; Fig. 2*C*) is suggestive. Furthermore, the occurrence of χ-like sequences (CCWCCWGC) in c-*myc* which have been causally related to chromosomal translocations involving the protooncogene *bcl-2* (30) is also noted, but its significance remains unclear.

Time and Origin of t(12;15). Preneoplastic proliferative foci of plasma cells have been identified morphologically in oil granulomatous tissues, but most of these occur beyond day 75 after injection of pristane. The surprising finding in this study was the presence of t(12;15)-specific PCR junction fragments in some sectors of the mesenteric oil granulomas of BALB/c mice 30 days after pristane. This provides compelling evidence that c-*myc* activating chromosomal translocations are a very early if not initiating step in plasma cell tumor formation. The restricted location of these cells to discrete sectors of the mesentery suggests that focal expansion of t(12;15)-positive cells, rather than diffuse infiltration, occurs at early stages of plasmacytomagenesis. The remarkably large number of recombinations at 30 days (8/20 mice, 40%) suggests, however, that many of these translocated cells do not successfully evolve into PCTs and that chromosomal translocations are not the limiting step in BALB/c plasmacytomagenesis. Subsequent events appear to be es-

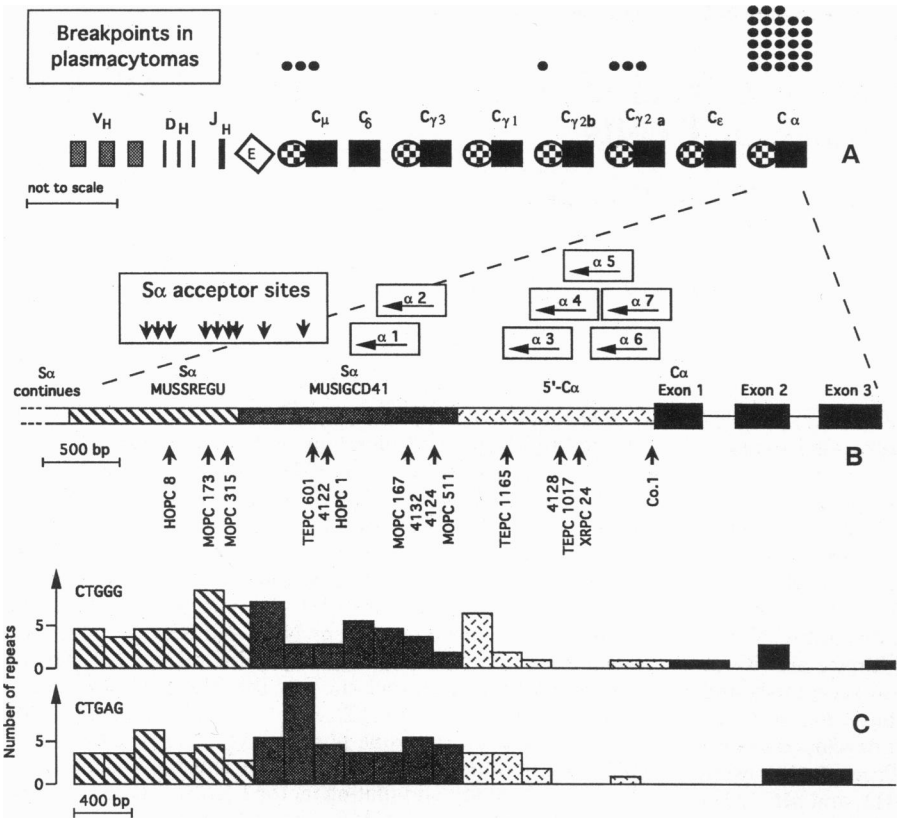

FIG. 3. (*A*) Molecular schema of the organization of the murine IgH gene locus. The V_H (variable region) genes, D_H (diversity region), and J_H (joining region) genes, the switch regions, and the C_H genes are represented by shaded boxes, vertical lines, circles, and filled boxes, respectively. The 5′ immunoglobulin enhancer is depicted by an open diamond. Dots above C_H genes represent individual murine plasmacytomas with molecularly characterized t(12;15)s that utilize the indicated switch region for juxtaposition to c-*myc*. (*B*) Diagram of the murine IgH_α locus—consisting of S_α, 5′-C_α, and C_α—and mapping of chromosomal breakpoints in that region. The extended S_α region represents the aligned GenBank entries MUSSREGU and MUSIGCD41. The fact that S_α continues further upstream (11) is illustrated. Complementary sites for primers α1–7 are also given (see Table 1). The distribution of translocation breaksites (↑) of t(12;15)s is compared with that of physiologic switch recombination (S_α acceptor) sites (↓) to C_α which are indicated on the basis of published data (11). The compilation of all published S_α acceptor sites indicates, however, that S_α recombination sites also occur 3′ of the box indicated (16), but not in 5′-C_α. (*C*) Frequency distribution of the S_α pentamers CTGAG and CTGGG along IgH_α. Shading of bars matches that in map *B*.

sential for the development of a neoplastic state. The results presented here add to the list of PCR-detectable protooncogene-activating events that occur early in neoplastic development of other hematological malignancies—e.g., chromosomal translocations t(14;18) that activate *bcl-2* in humans (31) and insertional mutagenesis in c-*myb* in mice (32).

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