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Genetics

Activation of the c-*myc* oncogene by the immunoglobulin heavy-chain gene enhancer after multiple switch region-mediated chromosome rearrangements in a murine plasmacytoma

(chromosome translocation)

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ABSTRACT **Presented is a detailed molecular analysis of the rearranged c-*myc* oncogene from ABPC45, an unusual plasmacytoma that was originally classified as translocation-negative. Previous data obtained by high-resolution chromosome banding suggested that this tumor was a member of a small group distinguished by the absence of rcpt (12;15) or (6;15) and further characterized by a band deletion near the c-*myc* locus on chromosome 15. However, genomic Southern blotting and analysis of the cloned oncogene in the present study reveal that (*i*) chromosome 12 sequences lie 365 base pairs 5′ of the rearranged c-*myc*; (*ii*) this DNA consists of immunoglobulin α switch region and 5′ immunoglobulin μ switch region sequences that are rearranged in an aberrant fashion; and (*iii*) the immunoglobulin heavy-chain gene enhancer element now resides ≈2.5 kilobase pairs 5′ of c-*myc*. We infer from these and other data that the rearrangement of c-*myc* in ABPC45 occurred via a multistep switch region-mediated process and that a reciprocal translocation has indeed taken place. Unlike many other plasmacytomas, this event did not interrupt the normal c-*myc* transcription unit. Rather, disruption of gene regulation is manifested in part by a change in relative usage of the two promoters normally used by the unrearranged gene. In contrast to several of its counterparts in Burkitt lymphomas, DNA sequence analysis of the translocated c-*myc* gene of ABPC45 reveals that it has not acquired point mutations in the noncoding first exon. These results strongly imply that a *cis*-acting regulatory element normally located 5′ of exon 1 is lost and that heavy-chain constant region or enhancer sequences exert similar *cis* effects on translocated c-*myc* loci.**

Plasmacytomas induced in BALB/c or NZB mice frequently possess rcpt (12;15) or (6;15). These translocations occur near the c-*myc* oncogene on chromosome 15 and juxtapose this gene and the immunoglobulin heavy-chain (IgH) constant region (C_H) locus on chromosome 12 or the κ light-chain locus on chromosome 6 (1, 2). The (12;15) and (6;15) translocations are termed typical and variant, respectively, based on their relative frequencies among tumors studied. It is generally accepted that activation of the c-*myc* gene as a result of its translocation to the immunoglobulin gene loci contributes to the development of malignant phenotype in tumors of the B-cell lineage, although the precise role of this gene in the transformation process as well as its function in normal cells remain to be elucidated.

In those tumors that have undergone rcpt (12;15), the c-*myc* gene is frequently broken—i.e., recombination has occurred within the gene (3–5). Breakage of this type does not alter the amino acid coding potential of c-*myc* because the breaks occur within the noncoding first exon or first intron. Although the gene's two normal promoters are removed by such recombination, production of truncated transcripts containing the two protein coding exons occurs from normally silent promoters in the intron (5). These observations led to proposals that c-*myc* expression was normally under negative regulation by first exon sequences and that removal of these putative control elements by gene breakage and translocation leads to constitutively deregulated expression (6, 7). It has also been proposed that the instability of full-length c-*myc* mRNA *in vivo* is related to the presence of first exon sequences (8). Thus, it is possible that truncated transcripts are not subject to normal post-transcriptional control. These hypotheses tend to be supported by the observation that c-*myc* exon 1 has undergone extensive mutation in several translocated but unbroken genes cloned from Burkitt lymphomas (9). More recently, however, examples of retroviral insertion 5′ of c-*myc* (10) and variation in a pattern of DNase I-hypersensitive sites in the 5′ flanking regions of c-*myc* in various genomic contexts (11) have been described. These results suggest a potential role for regions 5′ of c-*myc* in transcriptional regulation.

In this communication, we report the cloning and extensive molecular analysis of the rearranged c-*myc* gene from ABPC45, a plasmacytoma induced by pristane and Abelson murine leukemia virus (12). This tumor lacks cytogenetically detectable translocation, but it has undergone a hemizygous interstitial deletion of chromosome 15 (12). We found, however, that a series of switch (S) region-mediated recombinations has occurred between chromosomes 12 and 15, leaving the IgH enhancer (13, 14) ≈2.5 kilobase pairs (kbp) 5′ of c-*myc*. Alteration of c-*myc* transcriptional control is reflected by a change in the normal pattern of promoter usage. DNA sequence analysis shows that the translocated gene has not undergone mutations in the first exon, although several point mutations may exist in the 5′ flanking region.

MATERIALS AND METHODS

Molecular Cloning. A 14.8-kbp *Eco*RI fragment containing the rearranged c-*myc* gene of ABPC45 was partially purified by preparative agarose electrophoresis of restricted genomic DNA and cloned in the bacteriophage vector EMBL 4 (15).

DNA Blotting and Sequencing. Genomic DNAs were prepared and analyzed by Southern blotting as described (16).

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Abbreviations: bp, base pair(s); kbp, kilobase pair(s); IgH, immunoglobulin heavy chain; C_H, heavy-chain constant region; S, heavy-chain switch region.

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Sequencing was accomplished by the dideoxy-chain termination method, using M13 vectors (17).

S1 Nuclease Mapping. A clone consisting of a 700-base-pair (bp) *Eco*RI*/*Bam*HI fragment of c-*myc* 5' flanking and exon 1 sequences (18) and a 394-bp *Bam*HI/*Sst* I fragment from exon 1 was constructed in M13 mp10. This clone was used to prepare a uniformly labeled single-stranded probe for S1 nuclease mapping (5). Samples typically contained 2×10^4 cpm of probe and 2 μg of poly(A)$^+$ RNA; they were prepared and analyzed as described (18).

RESULTS

Multiple S Region Recombinations Contributed to the ABPC45 c-*myc* Rearrangement. The 14.8-kbp *Eco*RI fragment containing the rearranged c-*myc* gene of ABPC45 was cloned from genomic DNA. Fragments of DNA representing the gene's 5' flanking region were subcloned and used to probe Southern blots of DNAs from somatic cell hybrids containing defined mouse chromosomes on a hamster background (16). Contrary to our expectations, we found that this portion of the clone contained chromosome 12-specific DNA. Furthermore, the chromosome 12 DNA was determined to be composed of two segments that were not in germ-line context with respect to each other (data not shown).

Initial DNA sequence data obtained from the ABPC45 c-*myc* clone strongly suggested that immunoglobulin α S region (S_α) sequences were involved in this rearrangement, based on the prevalent repeating unit of the DNA (19). Subsequent comparison with germline S_α sequence allowed us to pinpoint the recombination site $\approx$365 bp 5' of exon 1 (Figs 1 and 2). Comparison of the ABPC45 sequence with previously published immunoglobulin μ S region (S_μ) 5' (20) and S_α (19) sequences enabled us to identify the other IgH component in the rearrangement, and we defined the second recombination site (Fig. 2; position −1163). Inspection of the sequences near each recombination site shows that c-*myc* has recombined with S_α in a "head-to-head" (5' to 5') orientation. S_μ 5' and S_α underwent "tail-to-tail" (3' to 3') recombination. Further sequence data obtained from the extreme 5' portion of the ABPC45 clone (Fig. 1) are in agreement with the germ-line S_μ 5' sequence (data not shown).

Genomic Southern blotting shows that ABPC45 appears to have undergone a reciprocal chromosome translocation. For each enzyme shown in Fig. 3, the c-*myc* probe hybridizes to three bands. One of these bands in each digest represents a germ-line copy of c-*myc* (*Eco*RI, 22 kbp; *Bam*HI, 7.2 kbp; *Bgl* II, 1.6 kbp). Another band is the expressed (translocated) gene [*Eco*RI, 14.8 kbp, based on our clone; *Bam*HI, 4.8 kbp; and *Bgl* II, 4.7 kbp, both based on comigration with bands hybridizing to S_α and S_μ 5' probes (Figs. 3 and 4; data not shown)]. The remaining band in each digest would represent 5' flanking c-*myc* DNA that has persisted in the genome of this tumor by virtue of its having participated in a reciprocal translocation. As expected, a DNA probe consisting of $\approx$7 kbp of 5' flanking c-*myc* sequences hybridized intensely to this reciprocal fragment (data not shown).

The *Igh* Enhancer Lies 5' of c-*myc* in ABPC45. Although the c-*myc* clone shown in Fig. 1 does not contain the IgH enhancer, we were able to show by genomic Southern blotting that this regulatory element lies in the predicted position $\approx$2.5 kbp 5' of exon 1. As shown in Fig. 3, *Bam*HI and *Bgl* II digests of ABPC45 DNA yield fragments of 4.8 and 4.7 kbp, respectively, which hybridize to both c-*myc* and enhancer probes. *Eco*RI, which cuts the genomic DNA between the regions expected to hybridize to these probes, does not yield such a comigrating band.

Context of Other Ig C_H Genes in ABPC45. Fig. 4 shows the results of Southern blotting using IgH enhancer, C_μ, and C_α/S_α to compare ABPC45 and BALB/c liver DNAs. The enhancer probe (Fig. 4*A*) hybridizes to several bands in ABPC45 DNA, one of which represents a germ-line copy of the heavy-chain joining region. The other bands correspond to copies of the enhancer associated with the rearranged c-*myc* and the functionally rearranged C_α gene in this tumor (see also Fig. 4*C*, probed with C_α/S_α). Hybridization with a C_μ probe (Fig. 4*B*) indicates that this gene is present only in germ-line context in ABPC45. Hybridization with C_γ and S_γ probes revealed that these sequences are also in germ-line context only (data not shown).

Alternative Promoter Usage in ABPC45. Shown in Fig. 5 are the results of quantitative S1 nuclease mapping to determine the relative transcription from the two normal c-*myc* promoters. Bands of 515 and 352 bases represent protection of the probe by transcripts arising from the 5' and 3' promoters, respectively. ABPC45 was compared with BALB/c spleen and ABPC26, which, like ABPC45, was classified as translocation negative and appears to possess a band deletion on chromosome 15 (12). In contrast to ABPC45, however, ABPC26 has not been shown to have a detectable rearrangement 5' of c-*myc*. Densitometric tracings were derived from the autoradiographs shown in Fig. 5 and were used to calculate the ratios of promoter usage shown in Table 1. We find that the more 5' promoter (P1) is relatively favored in ABPC45 compared with the spleen control and ABPC26. This quantitative analysis demonstrates that c-*myc* mRNA levels in the two tumors are increased relative to spleen and that the presence of the IgH enhancer near c-*myc* in ABPC45 does not result in higher steady-state levels of this transcript than in a cytogenetically analogous tumor that appears to lack such a rearrangement.

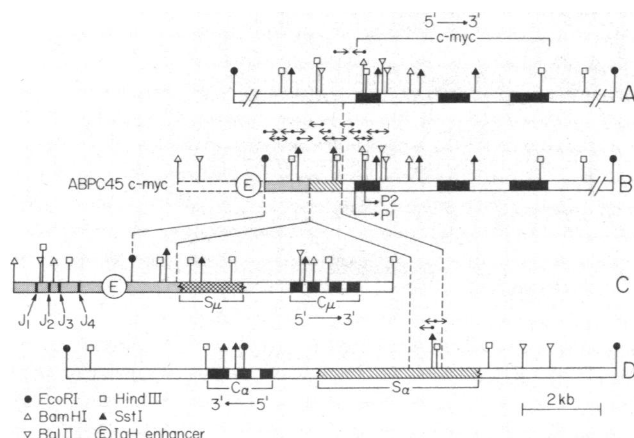

FIG. 1. Restriction map of ABPC45 c-*myc* rearrangement and its germ-line components. (*A*) Germ-line c-*myc*; (*B*) ABPC45 c-*myc* rearrangement; (*C*) germ-line joining region H chain, S_μ, and C_μ (*D*) germ-line S_α/C_α. Arrows indicate DNA sequencing strategy. The dashed segment 5' of ABPC45 c-*myc* shows DNA not present in our clone, which was mapped by genomic Southern blotting.

DISCUSSION

We have completed a detailed molecular characterization of the translocated c-*myc* gene from a tumor initially believed to be translocation negative. Data presented here leads us to conclude that an unusual two-step process occurred and that the first event was a rcpt **(12;15)** that involved S_α. As shown in Fig. 3, the putative reciprocal *Eco*RI fragment is 9.5 kbp, compared with a predicted size of 12.6 kbp if a precise rcpt **(12;15)** occurred (Fig. 1 *A* and *D*). Thus, we suggest that $\approx$3 kbp of DNA was deleted as a consequence of translocation, a commonly observed feature of these recombination events

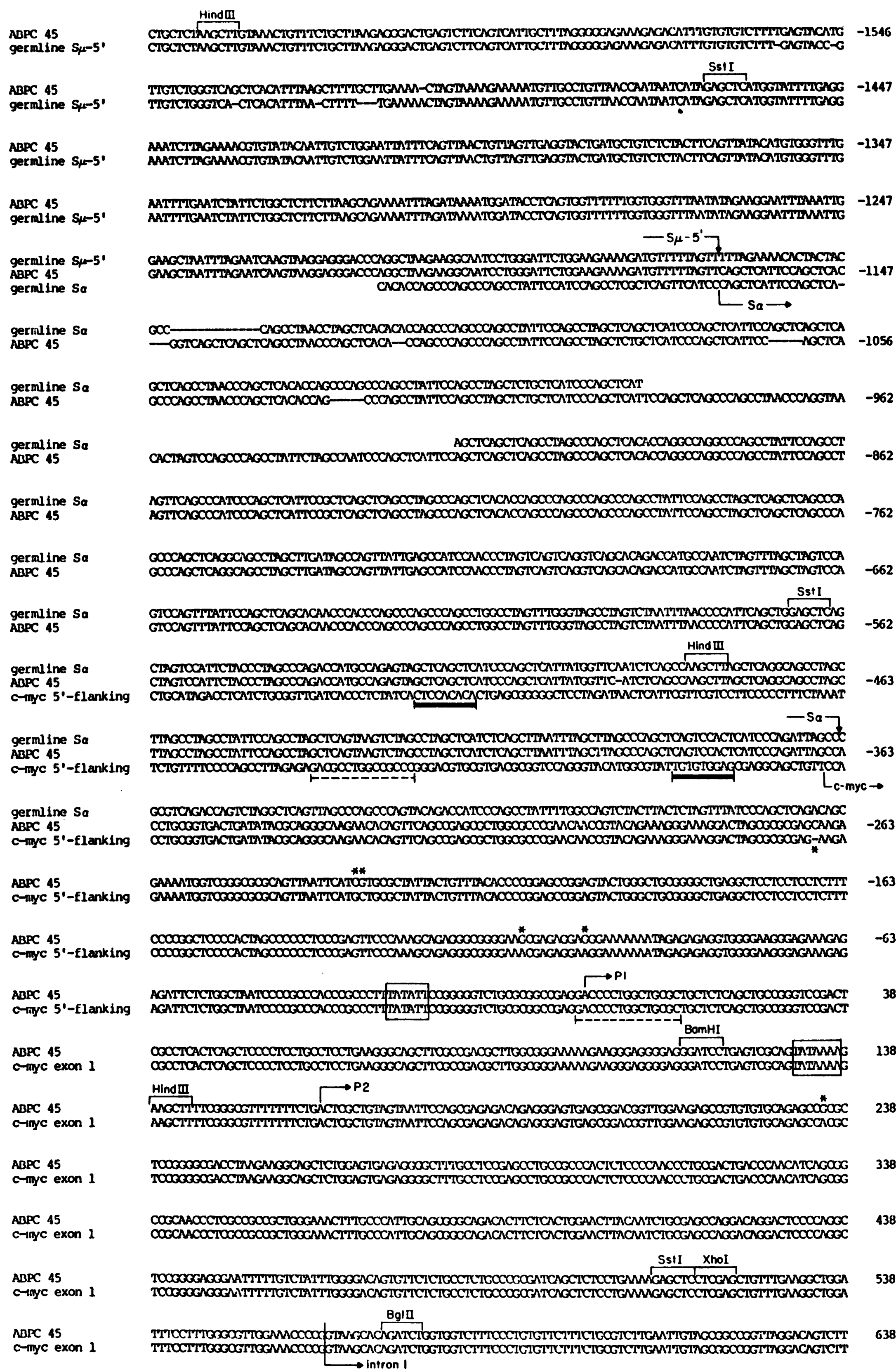

FIG. 2. Nucleotide sequence of the 5' flanking region and exon 1 of rearranged ABPC45 c-*myc*. The sequence is compared with its various germ-line components: S_μ 5' (20); S_α (ref. 19; this paper); c-*myc* 5' flanking (ref. 21; this paper); c-*myc* exon 1 (22). P1 and P2 indicate cap sites of transcripts arising from the two c-*myc* promoters ("TATA" sequences are boxed, P1 cap site is numbered +1). Dashes indicate gaps in one sequence with respect to another; the unmarked gap in germ-line S_α sequence reflects the lack of available data. Asterisks indicate possible differences between normal chromosome 15 and ABPC45 sequences. Recombination sites between S_μ 5' and S_α (position −1163) and between S_α and c-*myc* (approximate position −365) are indicated. Dashed underlines show two regions of DNA sharing a 12/15 sequence homology. Dark solid underlines indicate two segments of a 9-bp inverted repeat.

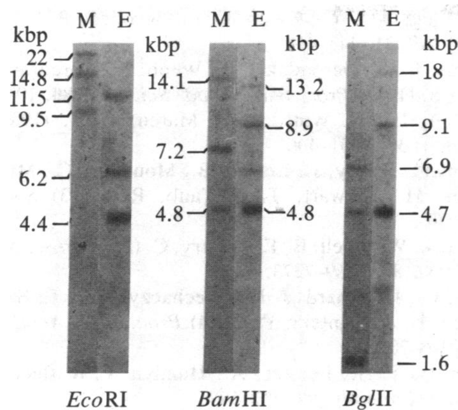

FIG. 3. Genomic Southern blot analysis of ABPC45. DNA was digested with the indcated enzymes and filters were probed with a c-*myc* probe (M) or an IgH enhancer probe (E). The c-*myc* probe was RB_2 (18), which spans the 5′ portion of exon 1 and ≈580 bp of germ-line 5′ flanking sequence. The enhancer probe was the 680-bp *Eco*RI/*Xba* I fragment containing the IgH enhancer (14) subcloned from pJ11 (23).

(18, 24). Furthermore, it is likely that this deletion occurred in the S_α region, because the narrowly defined c-*myc* 5′ probe is able to detect the putative reciprocal product. Our observations are therefore consistent with typical head-to-head recombination of c-*myc* with S_α that was followed by a second recombination event, either with the initial reciprocal chromosome or with another copy of chromosome 12. In mechanism, this may resemble a previously described multistep rearrangement in a plasmacytoma involving chromosomes 6, 12, and 15 (25). The second event occurred in a tail-to-tail manner, resulting in an inversion of the two IgH segments, which reside 175 kbp apart in germ-line DNA (26), with respect to each other and leaving the IgH enhancer ≈2.5 kbp 5′ of c-*myc*. It is possible that the presence of the band deletion on chromosome 15 is somehow related to this secondary rearrangement. Indeed, the detection of only cytogenetically normal copies of chromosome 12 in ABPC45 (12) suggests that the rearranged c-*myc* of ABPC45 resides on a "del 15" chromosome. Our data do not exclude other possibilities—for example, paracentric inversion of the C_H locus prior to invasion by c-*myc*. This seems unlikely, however, because it has no known precedent and Southern blotting does not reveal another product that would be predicted to arise from such an inversion (i.e., C_μ and C_α linked head to head) (see Fig. 4).

The positioning of the IgH enhancer 5′ of c-*myc* by an apparently S region-mediated mechanism is extremely interesting and unusual. There are examples of Burkitt lymphomas in which c-*myc* recombined 5′ of the enhancer (a region

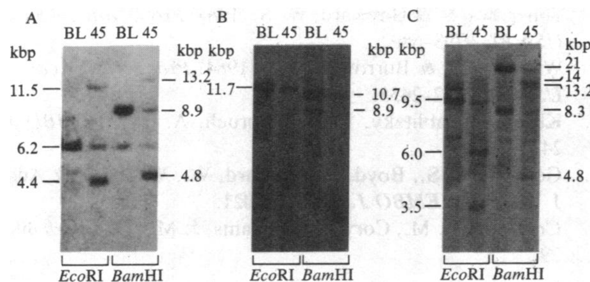

FIG. 4. Comparison of ABPC45 and germ-line IgH loci. BALB/c liver (BL) and ABPC45 (45) DNAs were digested by *Eco*RI or *Bam*HI and analyzed by genomic Southern blotting. Filters were probed with an IgH enhancer (see Fig. 3) probe (*A*), a C_μ cDNA (23) probe (*B*), or a probe containing S_α and the 5′ part of C_α (23) (*C*).

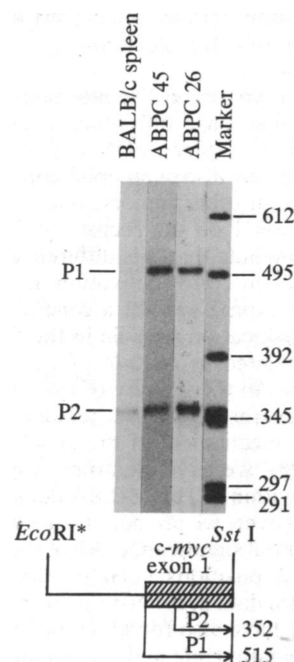

FIG. 5. S1 nuclease mapping of c-*myc* RNA from cells without cytogenetically detectable chromosome translocations. Protected fragments of 515 and 352 bases represent transcripts arising from P1 and P2, respectively. Sizes of marker fragments (φX174 *Hinc*II digest) are indicated. A map of the probe is shown below.

devoid of switch sequences) in a head-to-head manner (11, 27). Among plasmacytomas, there is one example, ABPC17, in which the IgH enhancer was inserted by a mechanism tail-to-head 5′ of c-*myc* (unpublished results). The possibility that a small segment of DNA carrying IgH enhancer was inserted 5′ of c-*myc* is precluded by our data, specifically the lack of a common *Eco*RI fragment in ABPC45 DNA that hybridizes to both the enhancer and c-*myc* probes (see Fig. 3).

DNA sequence analysis defined the chromosomal recombination site to be ≈365 bp 5′ of c-*myc* exon1 in ABPC45. This is in agreement with the clustering of translocation breakpoints 5′ of c-*myc* in other plasmacytomas and further supports our contention that a *cis*-acting regulatory element is located ≈350 bp of more 5′ of c-*myc* (unpublished results). The loss or disruption of this upstream region could be a major factor in the abnormal constitutive expression of c-*myc* in most plasmacytomas. Comparison of the ABPC45 S1 nuclease mapping data with that of another tumor that lacks a c-*myc* rearrangement suggests that transcriptional control is disrupted by translocation immediately 5′ of c-*myc* and that one manifestation of this is the relatively greater usage of P1. Promoter shifts of variable magnitude (P1/P2 = 0.85–2.6) have been found in ≈40% of plasmacytomas that have intact c-*myc* genes (unpublished results). This variation may be a consequence of multiple regulatory elements influencing the

Table 1. Relative c-*myc* promoter usage in plasmacytomas lacking cytogenetically detectable chromosome translocations

Source of RNA	c-*myc* context	P1/P2
BALB/c spleen	Germ line	0.43
ABPC26	NR*	0.42
ABPC45	5′ R*	0.75

*Based on Southern blotting data obtained from *Eco*RI digests (12). NR, not rearranged; 5′ R, rearranged 5′ of c-*myc*.

c-*myc* promoters differentially and in a manner dependent on the precise nature of a translocation (i.e., target DNA and distance from c-*myc*).

Analysis of the c-*myc* exon 1 sequence in this translocated gene reveals a single base difference compared to the sequence of a BALB/c spleen cDNA clone (22). The same solitary difference was also seen upon comparison of exon 1 sequences of several other tumors, one of which contained this portion of exon 1 on the reciprocal chromosome (18). Therefore, we conclude that this difference is unlikely to be a significant factor in c-*myc* activation in ABPC45 and that mutations within exon 1 are not a general feature of c-*myc* genes whose translocation sites lie in the 5′ flanking region.

A computer-assisted inspection of c-*myc* exon 1 and 5′ flanking sequences in the vicinity of the ABPC45 rearrangement reveals several structural features that may have relevance to the mechanism of the gene's activation. Like another group (28), we have identified what may be several point mutations within 300 bp of DNA flanking a translocated c-*myc* gene. However, we are not in a position at this time to assess their potential significance. All of these differences are in a region (Fig. 2; positions −100 to −300) whose sequence was obtained from data previously published by other investigators (21). We have also found a 9-bp inverted repeat just 5′ of the recombination site and a segment of DNA from the 5′ flanking region that shares a 12/15 homology with the extreme 5′ portion of exon 1 (see Fig. 2). The DNA between the latter two segments includes the translocation breakpoints of ABPC45 and five other plasmacytomas (unpublished results) and it is in a region that has substantial sequence homology with human c-*myc* (11, 21).

The fact that c-*myc* frequently recombines within the C_H locus in plasmacytomas but rarely involves the IgH enhancer suggests that some other property of these genes or their environment is capable of exerting a positive regulatory influence on the oncogene. Indeed, it has been shown that this enhancer is not an absolute requirement for efficient heavy-chain gene expression (29, 30). It also appears that an undefined *cis* defect may be capable of preventing expression of a functionally rearranged C_μ gene that is normal with respect to all previously described transcriptional regulatory elements (31). Taken together, these data could imply that the C_α gene that we suspect was the initial target of c-*myc* translocation in ABPC45 may have had (or subsequently acquired) some *cis* defect for which the IgH enhancer could compensate.

Note Added in Proof. The insertion of the IgH enhancer of 5′ of c-*myc* in ABPC17 has recently been published (32). Interestingly, the chromosome 15 breakpoint in this tumor is identical to that in ABPC45.

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