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Chromosome translocations clustered 5′ of the murine c-*myc* gene qualitatively affect promoter usage: implications for the site of normal c-*myc* regulation

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Five novel murine plasma cell (PC) tumors with chromosome translocations 350 – 500 bp 5′ of the first c-*myc* exon are described. The t(12;15)s of TEPC 1194, ABPC 33 and TEPC 1165 position the intact c-*myc* locus 5′ of the C_μ, $C_{\gamma 2a}$ and C_α IgC_H genes resepctively. In ABPC 17, the IgH enhancer element and adjacent switch (S_μ) sequences were found 5′ of the first c-*myc* exon while this enhancer is associated with the reciprocal products of the TEPC 1194, ABPC 33 and TEPC 1033 translocations. Quantitative S1 nuclease analyses demonstrate that the ratios of transcription from the two c-*myc* promoters (P_1 and P_2) are increased 4- to 7-fold in these five tumors. With the exception of TEPC 1165, (which contains a small deletion in exon 1), such increases in P_1:P_2 ratios appear to be manifested by a reduction in P_2 usage in comparison to other tumors without such promoter shifts. A survey of 27 additional PC and non-PC B lymphoid tumors and cell lines revealed that *myc* promoter shifts of this magnitude are unique to PC tumors with 5′-proximal translocations. We propose that (i) these clustered breakpoints identify a normal c-*myc* regulatory element located at least 350 bp 5′ of c-myc exon 1; (ii) the loss or disruption of this *cis*-acting upstream element and the linkage of c-*myc* to the IgC_H locus would result in abnormal expression of this oncogene in these as well as most other PC tumors.

Key words: chromosome translocations/murine plasmacytomas/ c-*myc* promoter shifts/5′ c-*myc* rearrangements

Introduction

Many murine plasmacytomas possess reciprocal translocations which link the coding exons of the c-*myc* proto-oncogene with the immunoglobulin heavy chain (IgH) constant region (C_H) gene locus (reviewed by Klein, 1983; Perry, 1983). In most cases, this recombination results in the loss or disruption of the normal promoters and first c-*myc* exon and activation of aberrant, 'cryptic' promoters residing in the first c-*myc* intron (Shen-Ong *et al.*, 1982; Adams *et al.*, 1983; Stanton *et al.*, 1983; Bernard *et al.*, 1983; Keath *et al.*, 1984). The protein products encoded by broken *myc* genes are presumed to be qualitatively normal since the first *myc* exon is an atypically large, 562 bp, non-coding sequence (Stanton *et al.*, 1983; Bernard *et al.*, 1983; Stanton *et al.*, 1984a, 1984b). The normal *myc* allele retained in these tumors is transcriptionally inactive suggesting that the level of *myc* transcripts produced from the broken gene is abnormal for these differentiated cells (Adams *et al.*, 1983; Stanton *et al.*, 1983; Bernard *et al.*, 1983). Similar observations in Burkitt lymphomas (Nishikura *et al.*, 1983; Taub *et al.*, 1984a) have led to the proposal that this conserved non-coding sequence is essential for normal *myc* gene regulation and may function by binding a putative repressor factor (Leder *et al.*, 1983; Dunnick *et al.*, 1983; Rabbitts *et al.*, 1984).

Here, we describe a class of PC tumors whose chromosome breakpoints occur within 350 – 500 bp 5′ of c-*myc* exon 1. The rearrangements in each of these tumors dramatically shift the utilization of the two normal c-*myc* promoters in favor of the 5′ start site, P_1, which strongly implies that an essential site for normal c-*myc* regulation, residing at least 350 bp 5′ of exon 1, has been lost or disrupted in these tumors.

Results

S1 nuclease analyses of intact c-myc gene expression

C-*myc* expression was investigated in plasmacytomas with chromosome translocations which do not disrupt the normal c-*myc* transcription unit. The murine c-*myc* gene is normally transcribed from two initiation sites which are separated by 163 bp (Stanton *et al.*, 1983,1984a,1984b; Bernard *et al.*, 1983). S1 nuclease mapping was performed with a uniformly labeled exon 1 probe (mp-R*S10 in Figure 1) to assess the relative usage of these dual promoters in 18 different plasmacytomas possessing either the predominant 12;15 or the variant 6;15 translocation (Figure 1 and Table I). Seven out of 18 tumors have higher P_1:P_2 usage ratios: ABPC 60, ABPC 33, TEPC 1165, TEPC 1033, ABPC 17, TEPC 1194 and PC 3741 (Yang *et al.*, 1984, and Table I this report). Except for TEPC 1165, shifts in *myc* promoter ratios appear to be mostly a consequence of reduced P_2 usage since P_1 levels do not seem appreciably higher in these cases (Figure 1). None of 14 non-PC B lymphoid tumors and cell lines displayed such promoter shifts (Table II). Quantitative S1 nuclease analyses in Figure 1 demonstrate that, except for TEPC 1165 which expresses an unusually high level of smaller exon 1 transcripts, PC and non-PC lympoid tumors generally produce comparable levels of *myc* transcripts with some variation. However, all B lymphoid tumors examined produce more *myc* RNA than normal spleen or thymus. The five PC tumors with the highest promoter shifts are known to contain rearranged *myc* genes (Cory *et al.*, 1983; Mushinski *et al.*, 1983a; Ohno *et al.*, 1984 and this report). The remainder of this report identifies the sites of *myc* rearrangement in these five tumors and the non-chromosome 15 targets for four of these translocations.

ABPC 33, TEPC 1194 and TEPC 1033

Unique rearranged *Eco*RI fragments corresponding to the expressed *myc* genes in ABPC 33 and TEPC 1033 have been previously identified (Mushinski *et al.*, 1983a; Cory *et al.*, 1983) while only a 21.5-kb germ line sized *myc Eco*RI fragment was detected in TEPC 1194 (Mushinski *et al.*, 1983a). Chromosome breakpoints in these three tumors were localized by Southern blotting which employed two probes containing c-*myc* exon 1 and 5′-flanking sequences. Bands corresponding to the expressed *myc* genes in these three tumors are denoted by an M and their

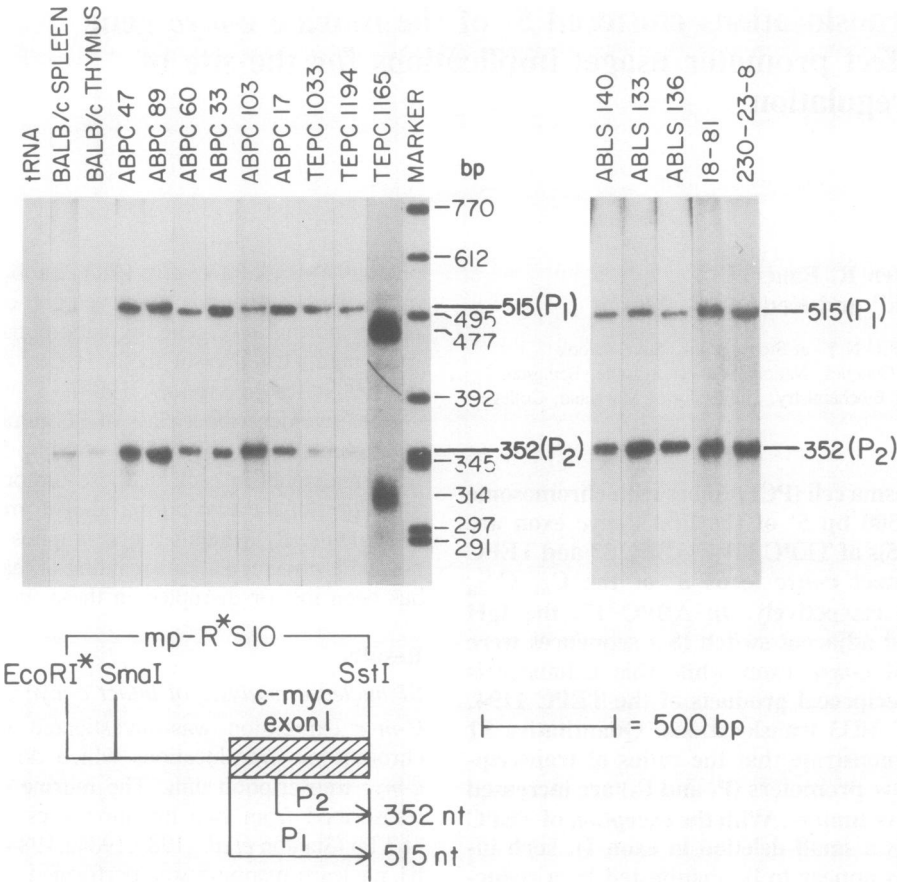

Fig. 1. S1 nuclease mapping of normal c-*myc* promoters in various transformed cell lines and plasmacytomas. 2 μg of poly(A)$^+$ RNAs were used for all samples. mp-R*S10 corresponds to a 1.1-kb segment of c-*myc* exon 1 and 5′-flanking sequences subcloned in M13 MP10 phage. Conditions for hybridizations, S1 nuclease and electrophoresis are discussed in Materials and methods. The expression of the P_1 promoter in TEPC 1165 was independently confirmed with a P_1-specific exon 1 probe which lacks exon 1 sequences downstream of P_2 (see R*B2 in Figure 6) (Stanton *et al.*, 1984a).

5′-reciprocal fragments by MR (Figures 1–4). M and MR bands were identified as follows; (i) an *Eco*RI*-*Sst*I probe consisting of 600 bp of c-*myc* 5′-flanking sequence and 515 bp of exon 1, pR*S10 (Figure 2), (Yang *et al.*, 1984) hybridized strongly to unrearranged c-*myc* and M fragments but less intensely to MR bands in most restriction digestions (see *Eco*RI data in Figure 2); (ii) a *Sma*I-*Bam*HI fragment, mp-SmB2 (Figure 2), containing 426 bp of c-*myc* 5′-flanking sequences and the first 118 bp of exon 1 hybridized to germ line and M bands but not to MR fragments in all restriction digestions (see *Bgl*II data in Figure 3). These DNA probes would be expected to hybridize to the expressed *myc* bands since they were not broken by translocation in these three tumors. As shown for the *Bgl*II digestions in Figure 3, pR*S10 hybridized to rearranged M and MR bands while mp-SmB2 only detected the M bands. The 1.75-kb *Bgl*II band common to all three tumors in Figure 3 corresponds to the germ line c-*myc* gene copy. These and analogous results with four other restriction enzymes (data not shown) demonstrate that the translocation breakpoints either reside between the *Eco*RI* and *Sma*I sites at the 5′ end of the pR*S10 (155 bp) or very near the 5′ end of mp-SmB2. Restriction maps of the 5′-flanking regions of these three expressed *myc* genes were assembled and compared with the germ line *myc* locus in Figure 6. We conclude that all three translocation sites reside 350–500 bp 5′ of c-*myc* exon 1.

The *myc* translocations in ABPC 33, TEPC 1194 and TEPC 1033 are also similar in that their 5′ MR bands are linked to the IgH enchancer element which has been previously identified (Gillies *et al.*, 1983; Banerji *et al.*, 1983; Neuberger, 1983). This was confirmed by employing an enhancer-specific probe (pj11 in Figures 2 and 3) in *Bam*HI and *Bgl*II digestions (see *Bgl*II data in Figure 3) and another probe consisting of sequences immediately 3′ of the enhancer (pj14RH in Figure 2) in *Eco*RI digestions. The *Eco*RI site at one end of both the pj11 and pj14RH probes is in fact the boundary between these two fragments and resides ~200 bp 3′ of the enhancer element in pj11 (Marcu *et al.*, 1980; Gillies *et al.*, 1983; Banerji *et al.*, 1983). A 700-bp *Eco*RI-*Xba*I fragment which contains the enhancer at the 3′ end of pj11 (Gillies *et al.*, 1983; Banerji *et al.*, 1983) was also observed to hybridize to MR bands in *Bam*HI and *Bgl*II digestions (data not shown). These results suggested that the M bands in these three tumors were linked in a 5′-5′ fashion with IgC_H genes as is usually the case with rcpt(12;15) which break the c-*myc* gene (reviewed by Klein, 1983; Perry, 1983). Southern hybridizations with C_H and S region probes demonstrated that $S_\mu C_\mu$ and $S_{\gamma 2a}$ are the respective targets for c-*myc* in TEPC 1194 and ABPC 33 (Figure 2). The rearranged and normal c-*myc* *Eco*RI fragments co-migrate in TEPC 1194 but are separable with other restriction enzymes (Figures 2 and 3). The rearranged *myc* gene in TEPC 1033 resides on a 27–33 kb *Eco*RI fragment (Cory *et al.*, 1983; Mushinski *et al.*, 1983a). Preliminary results indicate that $S_{\gamma 2a}$ and $C_{\gamma 2a}$ probes hybridize to a 27-kb *Eco*RI band in

Table I. Relative activity of normal c-*myc* promoters in plasmacytoid (PC) tumors.

	Translocation	c-*myc*-context	P1/P2
ABPC 47	rcpt(12;15)	NR	0.42
ABPC 52	rcpt(12;15)	NR	0.49
ABPC 89	rcpt(12;15)	NR	0.39
ABPC 60	rcpt(12;15)	NR	0.85
ABPC 65	rcpt(12;15)	NR	0.54
C6T TEPC 1156	rcpt(12;15)	NR	0.42
ABPC 33	rcpt(12;15)	5′R	1.71
TEPC 1165	rcpt(12;15)	5′R	2.62
TEPC 1194	rcpt(12;15)	5′R	1.79
TEPC 1033	rcpt(12;15)	5′R	1.49
ABPC 4	rcpt(6;15)	NR	0.48
ABPC 20	rcpt(6;15)	NR	0.38
ABPC 103	rcpt(6;15)	NR	0.18
ABPC 105	rcpt(6;15)	NR	0.50
ABPC 17	rcpt(6;15)	5′R	2.23
CAK TEPC 1198	inv(6;15)	NR	0.40
PC 3741	ND	NR	1.07
PC 7183	ND	NR	0.44

The cytogenetic analyses of these murine plasmacytomas have been described (Ohno *et al.*, 1984; Weiner *et al.*, 1984), except for C6T TEPC 1156, (F. Weiner, unpublished data). CAK TEPC 1198 contains a pericentric inversion of a Robertsonian 6;15 chromosome (Weiner *et al.*, 1984). PC 3741 and PC 7183 are NZB plasmacytomas of unknown karyotype. NR indicates no rearrangement detected in the 21.5-kb germ line c-*myc* *Eco*RI fragment except for TEPC 1194 where rearrangements were observed with other restriction enzymes (see text). The absence of a 5′ *myc* rearrangement in ABPC 4, 48, 60, 103 and PC 3741 was also confirmed by *Bam*HI digestion with an exon 1 probe (E. Remmers, unpublished data). Seven PC tumors with novel promoter shifts are indicated by arrows. P_1/P_2 ratios were derived from densitometric scans of autoradiographs of S1 mapping data (see Figure 1 for representative examples of these results). The sizes of the P_1 and P_2 derived exon 1 transcripts in TEPC 1165 are 38 nucleotides smaller than normal (see Figure 1).

TEPC 1033 (data not shown).

ABPC 17

The site of c-*myc* rearrangement in ABPC 17 is within 350 – 500 bp 5′ of the first *myc* exon. This was demonstrated in the same manner as shown above for ABPC 33, TEPC 1194 and TEPC 1033 (see ABPC 17 blots in Figures 2 and 3 and restriction map in Figure 6). Southern hybridizations with pj14RH (see *Eco*RI digestions in Figure 2) and pj11 (see *Bgl*II data in Figure 3) indicated that the ABPC 17 expressed *myc* gene recombined with S_μ sequences and that the IgH enhancer is 3 kb 5′ of the recombination site. Similar results were obtained in *Bam*HI digestions (data not shown). However, the *myc* rearrangement in ABPC 17 is unusual in several respects: (i) ABPC 17 has been shown to contain a 6;15 translocation (Ohno *et al.*, 1984) while our molecular results imply that the IgH enhancer on chromosome 12 is directly involved in this recombination; (ii) the ABPC 17 *myc* gene has recombined in a 3′-5′ (i.e., tail to head) fashion with the IgH enhancer (see Figure 6) while all other plasmacytoma *myc* rearrangements result in a 5′-5′ (i.e., head to head) linkage with a C_H gene (reviewed by Klein, 1983; Perry, 1983); (iii) pR*S10 and mp-SmB2 hybridizations of *Bam*HI- and *Bgl*II-digested ABPC 17 DNA revealed only one rearranged myc fragment corresponding to the expressed gene (see *Bgl*II data in Figure 3) but the expressed gene and a putative reciprocal fragment, (MR), are both observed in *Eco*RI, *Hind*III and *Sst*I digestions (see *Eco*RI data in Figure 2); and (iv) IgH enhancer probes not only hybridized to the expressed *myc* gene in *Bgl*II and *Bam*HI digestions (see *Bgl*II data in Figure 3) but also detect the putative (MR) band in an *Eco*RI digestion (Figure 4). These observations suggest that the M and (MR) bands are contiguous in ABPC 17 and do not result from a simple reciprocal translocation. Rather, our data are more consistent with the insertion of the enhancer and S_μ sequences 5′ of c-*myc* exon 1. For this reason, the additional rearranged *myc* band in ABPC 17 *Eco*RI digestions has been denoted (MR) (see Figures 2 and 4) for 'putative' *myc* reciprocal fragment.

Table II. Relative activity of normal c-*myc* promoters in pre-B and B lymphoid tumors and cell lines

	Translocation	c-*myc*-context	P1/P2
BALB/c spleen	–	NR	0.43
BALB/c thymus	–	NR	0.43
EA-3	–	NR	0.41
18-81	–	NR	0.35
230-23-8	–	NR	0.32
ABLS 133	–	NR	0.11
ABLS 5	–	NR	0.45
ABLS 8	–	NR	0.33
ABLS 1	–	NR	0.45
ABLS 103	–	NR	0.34
ABLS 106	–	NR	0.32
ABLS 19	–	NR	0.38
ABLS 127	–	NR	0.42
ABLS 136	–	NR	0.21
ABLS 140	–	NR	0.27
WEHI 231	–	NR	0.11

NR indicates no c-*myc* rearrangements in the 21.5-kb germ line c-*myc* *Eco*RI fragment. All tumors and cell lines are of BALB/c origin. EA-3 is an IL-3-dependent pre-B cell line (Palacios *et al.*, 1984). 18-81 and 230-23-8 are A-MuLV transformed pre-B cell lines (Alt *et al.*, 1981, 1982). ABLS samples are lymphosarcomas derived by innoculation with pristane and A-MuLV (Mushinski *et al.*, 1983b). WEHI 231 is a B lymphoma (Ralph, 1979). P_1/P_2 ratios were derived from S1 nuclease analyses of equivalent amounts of poly(A)$^+$ RNAs as described in Figure 1 and Table I.

The ABPC 17 *myc* restriction map in Figure 6 lends further support to some type of insertional mechanism. The IgH enhancer has been positioned ~200 bp 5′ of the *Eco*RI site residing between J_H elements and the C_μ gene in the BALB/c germ line (Gillies *et al.*, 1983; Banerji *et al.*, 1983, Figure 2). In the rearranged ABPC 17 *myc* gene, the IgH enhancer is found on a 0.6-kb *Bgl*II-*Eco*RI fragment, 3.0 kb 5′ of the c-*myc* exon 1. This *Bgl*II site does not reside near the IgH enhancer in the BALB/c germ line (Sakano *et al.*, 1980; Marcu *et al.*, 1980; Gillies *et al.*, 1983; Banerji *et al.*, 1983) and could not be generated by a V-D-J or D-J rearrangement since the J_H element closest to the enhancer (J_H4) is 0.8 kb upstream (i.e., 1.2 kb 5′ of the *Eco*RI site; Sakano *et al.*, 1983: Marcu *et al.*, 1980, and Figure 2). Furthermore, the *Bam*HI and *Bgl*II sites 5′ of both the rearranged ABPC 17 and germ line *myc* genes in Figure 6 are 6.3 kb apart suggesting that this region consists entirely of 5′-flanking c-*myc* DNA in agreement with other genomic blotting data obtained with more 5′ c-*myc* probes (data not shown). It is conceivable that the improper resolution of the molecular intermediates in a 12;15 reciprocal exchange contributed to this atypical sequence topology. The mechanism for such an unusual recombination and its relationship to the 6;15 translocation in ABPC 17 remain to be elucidated by molecular cloning.

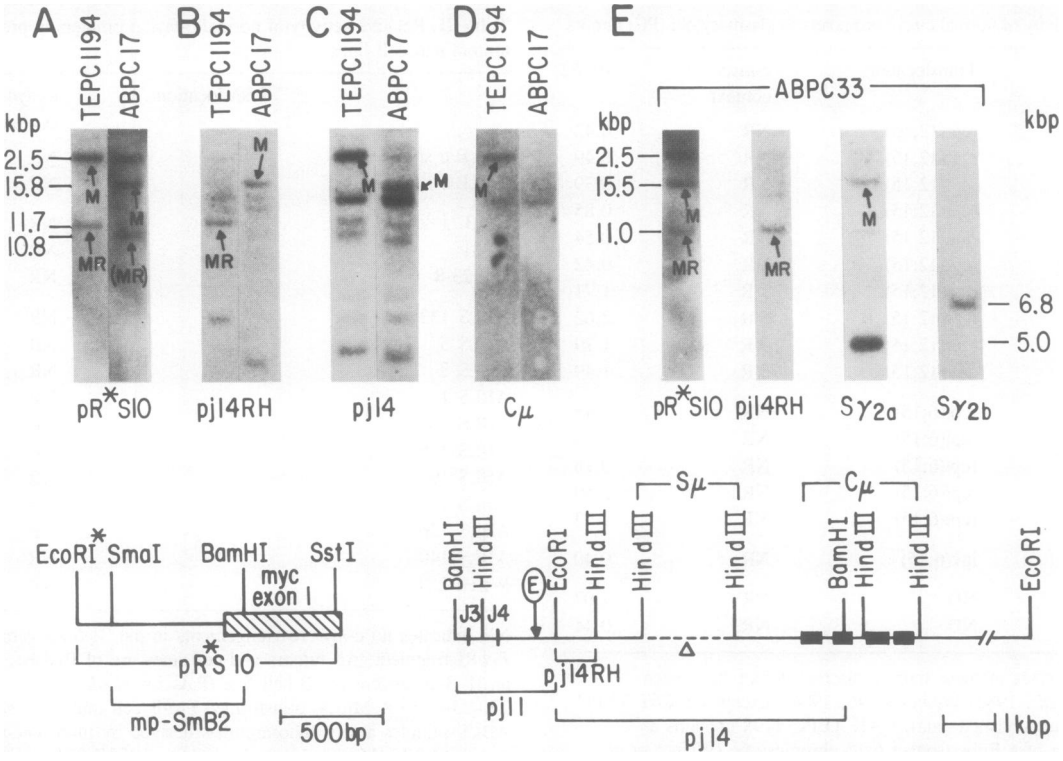

Fig. 2. Southern hybridizations of *Eco*RI digestions of ABPC 17, TEPC 1194 and ABPC 33. Each lane contains 7.5 μg of digested DNA. M and MR bands denote expressed *myc* and reciprocal *myc* fragments respectively. (MR) represents a putative reciprocal *myc* fragment (see text for explanation). $S_{\gamma 2a}$ and $S_{\gamma 2b}$ probes have been previously described (Harris *et al.*, 1983; Lang *et al.*, 1982). mp-SmB2 is a 544-bp DNA fragment cloned in M13 mp10. The Cμ probe is a Cμ cDNA clone and pj11, pj14RH and pj14 are subclones in pBR322 derived from a genomic clone of the BALB/c liver μ gene (Marcu *et al.*, 1980). The 21.5-kb band in ABPC 17 and ABPC 33 consists entirely of the germ line *myc* locus while this fragment co-migrates with the rearranged M band in TEPC 1194.

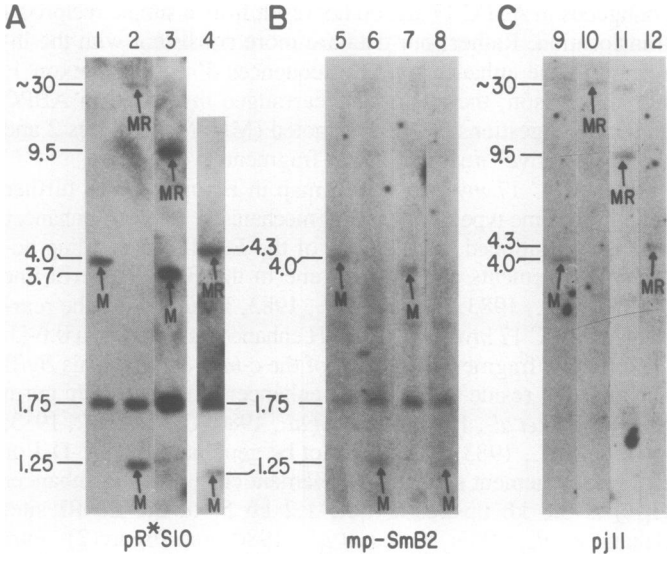

Fig. 3. Southern hybridization of *Bgl*II digestions of ABPC17 (**lanes 1,5,9**), ABPC 33 (**lanes 2,6,10**), TEPC 1194 (**lanes 3,7** and **11**) and TEPC 1033 (**lanes 4,8** and **12**). M and MR bands are indicated by arrows. A description of the probes is presented in Figure 2.

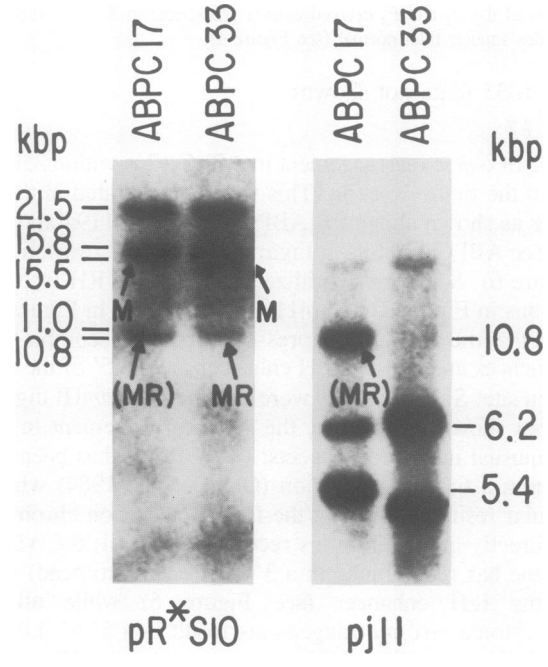

Fig. 4. Comparative Southern hybridizations of *Eco*RI-digested ABPC 17 and ABPC 33 DNAs with pR*S10 and pj11 (enhancer) probes. See diagram of DNA probes in Figure 1. M, MR and (MR) bands are denoted by arrows.

TEPC 1165

The TEPC 1165 *myc* gene is the only known example where a secondary mutation has radically affected the structure of the first c-*myc* exon in a plasmacytoma. S1 nuclease protection experiments indicated that 38 nucleotides of exon 1 sequence were absent from P_1 and P_2 transcripts in TEPC 1165 (Figure 1). These abnormal sized exon 1 bands are 10–20 times more intense than any of the normal sized transcripts in other tumors (Figure 1). These results agree with an earlier Northern blot analysis which

demonstrated that TEPC 1165 expressed high levels of abnormal sized *myc* RNAs (Mushinski *et al.*, 1983a). Genomic Southern blots (data not shown) and restriction analyses of the TEPC 1165 *myc Eco*RI fragment cloned in Charon 30 phage have independently confirmed that a small deletion (~200 bp) has removed the junctional region between the first exon and intron (see restriction map in Figure 6). The loss of the splice donor signal could also in part explain the predominant 3.5-kb *myc* RNA species originally detected in TEPC 1165 (Mushinski *et al.*, 1983a). Genomic Southern blots suggested that the C_α gene was involved in a 5′-5′ linkage with c-*myc* in TEPC 1165. However, the precise location of the translocation site was difficult to assess since a reciprocal product was not observed with the pR*S10 probe (data not shown). Therefore, the TEPC 1165 *myc* genomic clone was submitted to single and double restriction digestions which were hybridized to pR*S10, S_α and C_α probes (Figure 5). A restriction map of the TEPC 1165 *myc* gene was assembled from both genomic DNA and clone analyses (Figure 6) and it confirms the head to head joining of c-*myc* and the C_α gene in the region 350–500 bp 5′ of *myc* exon 1.

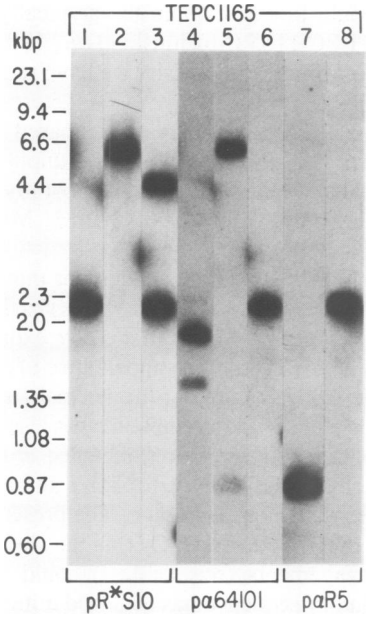

Fig. 5. Southen hybridizations of TEPC 1165 expressed *myc* genomic clone identifies the c-*myc* target as the Cα gene. A 15.5-kb *Eco*RI fragment corresponding to the expressed *myc* gene in TEPC 1165 was purified by agarose gel electrophoresis and cloned in the Charon 30 phage vector (Rimm *et al.*, 1980) (S. Bauer and J.F. Mushinski, unpublished data). The genomic clone was submitted to the following double digestions: *Eco*RI + *Xba*I (**lanes 1** and **4**), *Eco*RI + *Xho*I (**lanes 2,5** and **7**) and *Eco*RI + *Hind*III (**lanes 3,6** and **8**). pα64101 consists of a 4.5-kb *Hind*III-*Eco*RI fragment which contains S_α and C_α sequences (Stanton and Marcu, 1982). pαR5 is a 960-bp *Eco*RI fragment representing the 5′ portion of a C_α cDNA clone (Early *et al.*, 1979).

Discussion

Expression of intact c-myc genes in plasmacytomas

We have employed quantitative S1 nuclease analysis to compare the steady-state levels and structures of c-*myc* RNAs in 18 PC and 14 non-PC B lympoid tumors and cell lines with intact c-*myc* genes. Two general conclusions can be derived from these results: (i) a higher *myc* promoter ratio is a unique feature of ~40% of PC tumors with intact *myc* genes; and (ii) elevated levels of normal sized c-*myc* transcripts (compared with normal spleen and thymus) are found in a large survey of PC and non-PC B lymphoid tumors demonstrating that abnormal amounts of *myc* RNAs are a common characteristic of transformed B cells. The former observation contrasts with findings on translocated *myc* genes in Burkitt lymphomas where c-*myc* promoter shifts have been detected for all intact *myc* genes regardless of the site of chromosome translocation (Taub *et al.*, 1984a, 1984b; Hollis *et al.*, 1984). In addition, as shown clearly in Figure 1, the *myc* promoter shifts detected in four PC tumors (ABPC 33, ABPC 17, TEPC 1033, TEPC 1194) are largely manifested by a reduction in P_2-derived *myc* RNAs which accumulate at steady-state. We consider it unlikely that such promoter shifts are a consequence of differential stabilities of P_1 versus P_2 initiated RNAs.

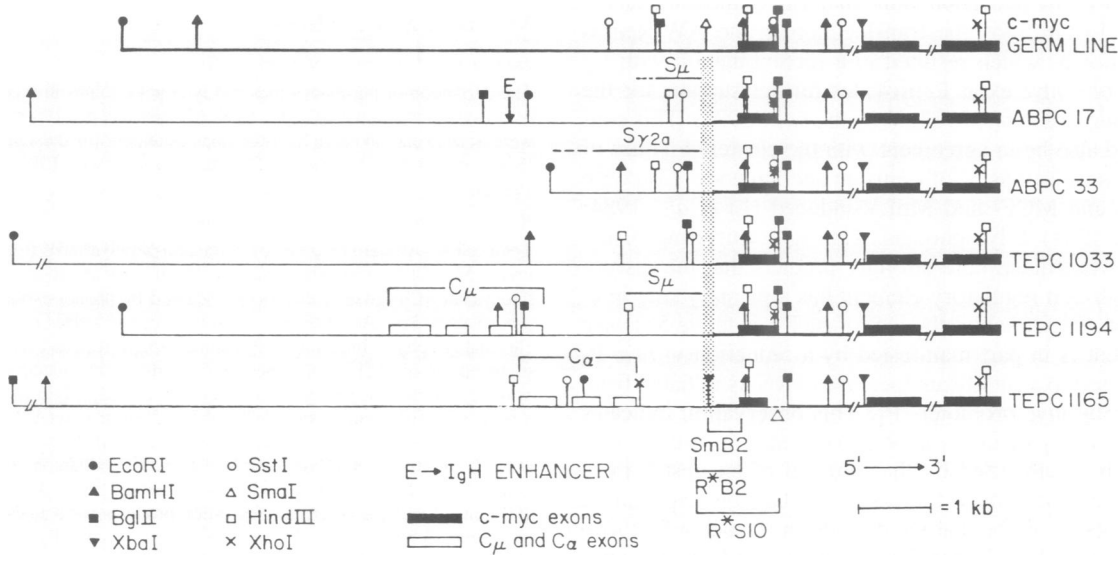

Fig. 6. Restriction maps of expressed *myc* genes in plasmacytomas with 5′ c-*myc* rearrangements. Only the individual restriction sites which map closest to the first c-*myc* exon are indicated. The TEPC 1165 map was assembled from Southern hybridizations on genomic DNA and a genomic clone of the rearranged *myc Eco*RI fragment. DNA probes employed in Southern blotting experiments are indicated below the maps. The rearranged *Eco*RI and *Bgl*II fragments in TEPC 1033 and 1165 are ~27 and ~30 kb respectively. The stippled bar indicates the approximate boundaries of the translocation sites determined from Southern blotting data with the indicated probes.

In human cell lines, the stabilities of P_1 and P_2 myc RNAs have been found to be comparable (Dani *et al.*, 1984). We have recently confirmed this later finding in murine pre-B lymphomas and plasma cell tumors with intact myc genes (M. Piechaczyk *et al.*, unpublished results).

The PC tumor with the highest P_1:P_2 ratio, TEPC 1165, also expressed high levels of abnormal sized exon 1 transcripts. The presence of only two truncated exon 1 transcripts demonstrates that the normal *myc* allele is transcriptionally silent in TEPC 1165 (Figure 1), in agreement with observations in other plasma cell tumors (Adams *et al.*, 1983; Stanton *et al.*, 1983; Bernard *et al.*, 1983). The normal *myc* alleles in the remaining 17 PC tumors examined may also be transcriptionally silent, but this could not be directly demonstrated since the exon 1 derived transcripts from the normal and translocated alleles would co-migrate in these cases.

Clustered translocations predict a site for normal c-myc control

Five of seven plasmacytomas with abnormal promoter ratios were found to contain chromosome translocation sites clustered within 350–500 bp 5′ of the first c-*myc* exon (Figure 6). All five of these translocations involved C_H gene S regions. Four out of five cases represent 5′-5′ (head to head) linkages to one of three S_HC_H targets ($S_\mu C_\mu$, $S_{\gamma 2a}$ and C_α) and are therefore somewhat analogous to the majority of PC tumors where translocations break the c-*myc* gene (reviewed by Klein, 1983; Perry, 1983). The ABPC 17 *myc* gene underwent a novel 3′-5′ (tail to head) recombination with S_μ and the IgH enhancer which may have resulted from an insertion mediated by a 12;15 translocation. These observations demonstrate that c-*myc* $P_2 \rightarrow P_1$ promoter shifts occur whether the target of the translocated *myc* gene is a C_H gene or the IgH enhancer. This also strongly supports the contention that C_H S regions play an integral role in such translocations in murine plasmacytomas even though neither the c-*myc* gene nor its 5′-flanking sequences possess appreciable homology to S regions (Cory *et al.*, 1983; Stanton *et al.*, 1984a; Gerondakis *et al.*, 1984; Marcu *et al.*, 1984).

We suggest that the removal or disruption of a regulatory element located at least 350 bp 5′ of exon 1 is the common mechanism for c-*myc* activation in the majority of these translocations. The 8;14 chromosome translocation in the AW-Ramos Burkitt lymphoma, which resulted in a recombination with C_μ ~340 bp 5′ of c-*myc* exon 1, provides further support for the hypothesis (Bernard *et al.*, 1983; Wiman *et al.*, 1984). This conclusion would also be in agreement with the clustered nature of MCF provirus insertions 5′ of c-*myc* in spontaneous (Corcoran *et al.*, 1984) and MCF- and MuLV-induced (Li *et al.*, 1984; Selten *et al.*, 1984) T lymphomas. The tight clustering of the translocations examined here strongly predicts that the disruption of an upstream regulatory element has a primary role in c-*myc* activation. Remarkably, the loss of such a putative 5′ c-*myc* control element is in part manifested by a reduction in *myc* P_2 usage as opposed to a significant increase in RNAs initiated from the more nearby *myc* promoter, P_1. This observation indicates that normal *myc* P_1:P_2 ratios may be regulated in a concerted manner which is subverted by the removal of an upstream c-*myc* control element in these tumors. One plausible explanation for our findings would be that the disruption of such a 5′ c-*myc* flanking sequence alters P_1 regulation which simultaneously has a negative effect on P_2 usage. This hypothesis is testable through gene transfer experiments.

Secondary alterations or losses of *myc* exon 1 sequences (see TEPC 1165 in this report) need not be essential for the initial activation event in plasmacytomas but may provide some additional selective advantage. It has recently been shown that the normal sized c-*myc* mRNAs in a variety of cell lines are extremely unstable (10–15 min half life) and that *myc* mRNA stability can be potentiated in some cases by protein synthesis inhibitors (Dani *et al.*, 1984). Alterations in exon 1 and/or the presence of intron 1 sequences in *myc* mRNAs may have a radical effect on mRNA stability, and could in part explain the atypically high level of *myc* RNAs in TEPC 1165 (Mushinski *et al.*, 1983a) (Figure 1). In further support of this hypothesis, we have recently observed that the stabilities of truncated *myc* RNAs expressed by broken *myc* genes are significantly enhanced compared with their normal counterparts (M. Piechaczyk *et al.*, unpublished results) which may in part explain the higher levels of myc RNAs in this class of plasmacytomas (Marcu *et al.*, 1983; Mushinski *et al.*, 1983a).

A *cis*-acting 5′ c-*myc* regulatory element may be the site of action of a repressor which has been hypothesized to control *myc* expression in interacting with the first c-*myc* exon (Leder *et al.*, 1983; Dunnick *et al.*, 1983). The appearance of a strong DNase I hypersensitive site 1.8 kb 5′ of the normal (transcriptionally silent) c-*myc* allele in a Burkitt lymphoma has also been attributed to the action of such a repressor (Siebenlist *et al.*, 1984) The existence of such a DNase I hypersensitive site near the normal murine c-*myc* gene remains unknown. The presence of *myc* promoter shifts in PC 3741 and ABPC 60 in the absence of obvious 5′ c-*myc* rearrangements (see Table I) could imply that this upstream regulatory sequence has acquired a more subtle structural alteration or that the translocation has disrupted a secondary locus involved in c-*myc* regulation. The existence of an additional class of PC tumors with normal promoter ratios and no detectable 5′ c-*myc* rearrangements (11 examples in Table I) imply that more distant, but cytogenetically analogous translocations may modulate *myc* expression by an independent mechanism. It is also conceivable that these distant translocations do not have a *cis* effect on c-*myc* expression, but alter the expression of other transformation associated gene(s) which may reside in close proximity to the c-*myc* locus.

Materials and methods

Tumors and Southern blot hybridizations

The derivation of plasmacytomas and lymphosarcomas in this study have been described elsewhere (Ohno *et al.*, 1984; Mushinski *et al.*, 1983b). Genomic DNAs were isolated and Southern hybridizations were performed essentially as described (Southern, 1975; Lang *et al.*, 1982; Marcu *et al.*, 1980, 1983; Mushinski *et al.*, 1983a).

RNA isolation and S1 nuclease mapping

Total cell RNAs were prepared by extraction of pulverized, frozen tissues in 6 M urea/3 M LiCl (Auffray and Rougeon, 1980) or by extraction with guanidinium thiocyanate (Chirgwin *et al.*, 1979) followed by phenol extraction and ethanol precipitation. Poly(A)$^+$ RNAs were isolated by two cycles of oligo(dT)-cellulose chromatography. Uniformly ^{32}P-labeled, single-stranded DNA probes were prepared from M13 subclones and purified by electrophoresis in 6% polyacrylamide/8 M urea gels (Stanton *et al.*, 1983). Hybridizations of 2 x 10^4 c.p.m. of purified single-stranded probe to 2 μg of poly(A)$^+$ RNAs were performed in 80% formamide (Fluka)/0.4 M NaCl/0.4 M Pipes, pH 6.4/1 mM EDTA at 58°C for 10–15 h. (Casey and Davidson, 1977; Stanton *et al.*, 1984a, Yang *et al.*, 1984). S1 nuclease digestions were carried out at 37°C for 30 min with 100 units of enzyme (BRL) and digestion products were analyzed on 6% polyacrylamide/8 M urea gels. The relative usage of P_1 and P_2 *myc* promoters were calculated from densitometric scans of autoradiographs.

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ABPC-17 tumor cells have recently been cytogenetically re-examined and found to contain an inv(12;15) as opposed to the original rcpt(6;15) classification (F.Weiner, personal communication). An independent study on the *myc* gene rearrangement in ABPC 17, which concurs with and extends our data, was published after the submission of this manuscript (Corcoran,L.M., Cory,S. and Adams,J.M. (1985) *Cell*, **40**, 71-79). In addition, we have also found that the c-*myc* rearrangement in another tumor, ABPC 45, which contains a $P_2 \rightarrow P_1$ shift, occurred 361 by 5′ of a structurally normal c-*myc* first exon (Fahrlander *et al.* (1985) *Proc. Natl. Acad. Sci. USA*, in press).