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Chromatin structure of the murine c-*myc* locus: implications for the regulation of normal and chromosomally translocated genes

Paul D.Fahrlander, Marc Piechaczyk[1] and Kenneth B.Marcu

Department of Biochemistry, State University of New York at Stony Brook, Stony Brook, NY 11794, USA and [1]Laboratoire de Biologie Moléculaire, Université des Sciences et Techniques du Languedoc, 34060 Montpellier Cedex, France

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To assess possible alterations of c-*myc* transcriptional control in murine B-cell tumors, we have investigated the pattern of DNaseI hypersensitive sites in the gene's putative regulatory region and within the gene in a variety of genomic contexts. A number of such sites were found in several cell types, but none of these was detectable in a gene which was shown to be transcriptionally silent by the criterion of elongation of nascent transcripts in isolated nuclei. These results differ from those of a previous study, in which a DNaseI-hypersensitive site ~2 kb upstream of the gene was proposed to be associated with negative regulation of c-*myc* transcription in human cells. An analysis of DNA sequences presented here reveals that this region is highly homologous between mouse and human, suggesting that these upstream hypersensitive sites do not reflect species-specific regulatory elements. We also present data indicating that this hypersensitive site distinguishes the c-*myc* alleles in translocation-positive plasma cell tumors which lack c-*myc* rearrangement. Furthermore, we report the existence of hypersensitive sites within the gene. One of these appears to be associated with cryptic promoters that are employed only when the normal promoters are lost as a consequence of chromosome translocation. These results are discussed in the context of c-*myc* translocation and gene breakage and with respect to possible stage-specific regulation of the gene's transcriptional competence.

Key words: chromosome translocation/c-*myc*/DNaseI hypersensitivity/plasma cell tumor

Introduction

The c-*myc* proto-oncogene is the cellular homolog of the transforming gene of the myelocytomatosis virus. Although no specific role has been assigned to its protein product, it has been proposed that c-*myc* may function in the process of cell proliferation (Land *et al.*, 1983a, 1983b; Kelly *et al.*, 1983; Armelin *et al.*, 1984; Keath *et al.*, 1984; Persson *et al.*, 1984; Kaczmarek *et al.*, 1985). It appears that alteration of this gene's pattern of expression, either by gene amplification (Alitalo *et al.*, 1983; Little *et al.*, 1983; Schwab *et al.*, 1985), retroviral insertion (Corcoran *et al.*, 1984; Li *et al.*, 1984) or chromosome translocation may be an important step in the development of a variety of neoplasms.

In some tumors of the B-cell lineage (i.e., murine plasma cell tumors and human Burkitt lymphomas), c-*myc* is frequently rearranged *via* reciprocal chromosome translocation with the immunoglobulin heavy or light chain loci (for reviews, see Klein, 1983; Leder *et al.*, 1983; Perry, 1983; Marcu *et al.*, 1984a, 1984b; Klein and Klein, 1985). Among plasma cell tumors, translocation breakpoints have been described: (i) several hundred base pairs (bp) upstream of c-*myc* (Corcoran *et al.*, 1985; Fahrlander *et al.*, 1985; Yang *et al.*, 1985); (ii) at a large distance from the gene, such that no rearrangement of c-*myc* is detectable by genomic Southern blot analysis (Ohno *et al.*, 1984); or (iii) within the normal c-*myc* transcription unit (Shen-Ong *et al.*, 1982; Marcu *et al.*, 1983; Adams *et al.*, 1983; Stanton *et al.*, 1983). In the last case, truncated mRNAs are produced from normally silent promoters in the gene's first intron, while no transcripts of the normal (untranslocated) allele can be detected by Northern blot analysis of the steady-state mRNA pool (Adams *et al.*, 1983; Stanton *et al.*, 1983; Bernard *et al.*, 1983). Thus, disruption of normal c-*myc* transcriptional control in these tumors may be linked both to removal of the gene's normal promoters (as well as other proposed regulatory sequences in the flanking region) (Yang *et al.*, 1985) and to its juxtaposition with the immunoglobulin locus. Moreover, among those examples cited in (i) above, loss of normal regulatory sequences and the presence (in some cases) of defined *cis*-acting immunoglobulin elements (Corcoran *et al.*, 1985; Fahrlander *et al.*, 1985) is manifested by a shift in usage of the normal c-*myc* promoters in favor of the more upstream promoter (Yang *et al.*, 1985; Fahrlander *et al.*, 1985).

To investigate possible perturbations of c-*myc* transcriptional control in plasma cell tumors (PCTs) which have no obvious structural abnormalities in their c-*myc* loci [i.e., those mentioned in (ii) above and the untranslocated alleles of those in (iii)], we have examined the chromatin structure of c-*myc* and its flanking sequences. This was accomplished by mapping sites of increased sensitivity to DNaseI, the presence of which has been correlated with actively transcribed genes (for review, see Weisbrod, 1982), including human c-*myc* (Tuan and London, 1984). Unlike a previously published study of a Burkitt lymphoma (Siebenlist *et al.*, 1984), however, none of the murine cell lines we investigated had a DNaseI-hypersensitive site that appeared to be associated with repression of transcription. Furthermore, DNaseI hypersensitivity was consistently detected within the transcribed c-*myc* gene, in a region which corresponds to the cryptic promoters mentioned above, in a variety of cell types and regardless of the gene's context.

Results

Pattern of DNaseI hypersensitivity associated with unrearranged c-myc

We first attempted to define the pattern of DNaseI hypersensitive (DH) sites within and upstream of c-*myc* in cells which have no alteration of the gene's structure based on Southern blot analyses. Representative results obtained from several cell lines are shown in Figure 1. The c-*myc* gene in its unrearranged context resides on a 10.5-kb *Kpn*I fragment (Figure 1, c-*myc* parent band). When DNA prepared from DNaseI-treated nuclei was digested with *Kpn*I and Southern blots were probed with a c-*myc* probe

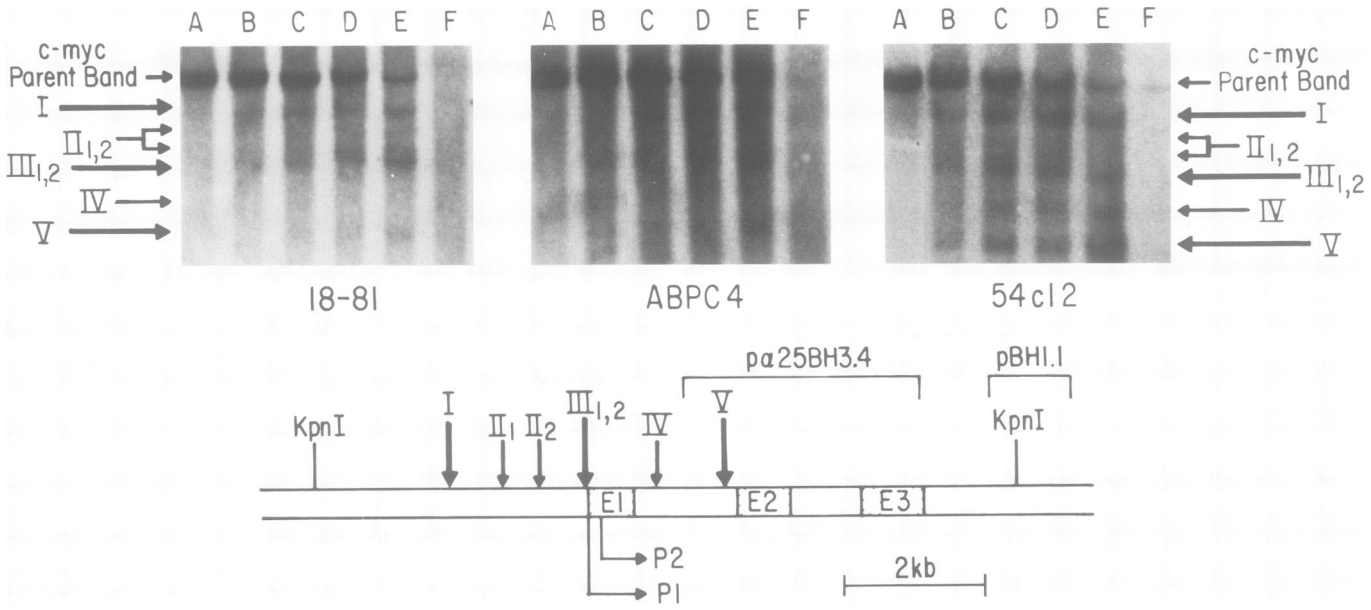

Fig. 1. DNaseI hypersensitivity within and upstream of unrearranged c-*myc*. DNA from DNaseI-treated nuclei was digested with *Kpn*I and analyzed by Southern blotting; filters were probed with pα25BH3.4. 18-81 is an A-MuLV pre-B lymphoma, ABPC4 is a plasma cell tumor with a rcpt(6;15), 54c12 is an A-MuLV transformed fibroblast line with an amplified c-*myc* locus. Sub-bands generated from the 10.5kb c-*myc* parent fragment by DNaseI treatment of chromatin are indicated and correspond to cleavage at the sites shown on the map. Levels of DNaseI used were as follows (units/2 × 10^7 nuclei): **lane A**, 0; **lane B**, 1.0; **lane C**, 2.0; **lane D**, 4.0; **lane E**, 8.0; **lane F**, 16. E1, E2 and E3 indicate the gene's three exons. P1 and P2 show the transcriptional start sites for the two c-*myc* promoters.

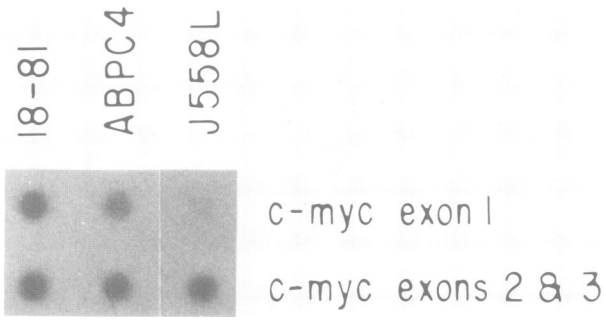

Fig. 2. Transcriptional activity of c-*myc* loci. Nascent transcripts were labeled in isolated nuclei (Schibler *et al.*, 1983) and hybridized to nitrocellulose filters on which excess DNA probes had been spotted (Piechaczyk *et al.*, 1984). The exon 1 probe was pR*S_{10} (Yang *et al.*, 1985) and the exon 2 and 3 probe was pMYC7.4 (Watt *et al.*, 1983). 18-81 and ABPC4 are described in Figure 1. J558L has a rcpt(12;15) which truncated c-*myc* (Gerondakis *et al.*, 1984).

(pα25BH3.4), several sub-bands were detected. These sub-bands were generated by specific cleavage of chromatin at the DH sites indicated in Figure 1. Five of these, I, II_1, II_2, III_1 and III_2, correspond to DH sites consistently found to be associated with transcriptionally active human c-*myc* (Siebenlist *et al.*, 1984; Dyson and Rabbitts, 1985). In addition, we have found two DH sites, IV and V, within the gene's first intron. The latter, which we found in all cells tested, presumably corresponds to a weak site found sporadically in some human c-*myc* genes (Siebenlist *et al.*, 1984). The positioning of all sites was confirmed by hybridizing the same filters with a 3′-flanking c-*myc* probe (see pBH1.1, Figure 1), which yields an identical pattern of hybridization and detects an additional downstream *Kpn*I fragment (data not shown).

Transcriptionally silent c-myc contains no hypersensitive sites

To compare DH sites in transcribed versus non-transcribed c-*myc* in cells containing chromosome translocations, it was necessary to study cell lines in which: (i) the translocated and normal alleles could be distinguished by Southern blotting, and (ii) the potential transcription units of the two genes were different, such that the two alleles could be distinguished at the RNA level. To accomplish this, we chose the plasma cell tumors MPC-11 and J558L, both of which possess rcpt(12;15) which occurred within the normal gene's first exon and produce truncated c-*myc* mRNA from cryptic intron promoters (Stanton *et al.*, 1983; Marcu *et al.*, 1983).

Although no transcription of normal c-*myc* (i.e., exon 1-containing RNA) can be detected in steady-state pools of J558L or MPC-11 mRNA (Stanton *et al.*, 1983. Marcu *et al.*, 1984a), we decided to determine whether the normal gene was in fact silent at the level of transcription. Figure 2 shows the result of an analysis in which run-on transcripts were labeled in isolated nuclei and hybridized to cloned DNA probes on nitrocellulose filters. When a c-*myc* exon 1 probe was used, a signal was present for 18-81 and ABPC4 (which produce normal-sized c-*myc* transcripts from unrearranged genes), whereas none was apparent above background for J558L. On the other hand, all three cell lines appear to have approximately equivalent amounts of transcription of c-*myc* exons 2 and 3. We conclude, therefore, that the lack of exon 1 transcripts in the steady-state mRNA pool faithfully reflects an absence of transcription of the normal allele in J558L and that this gene provides a valid model for a structural study of untranscribed c-*myc*. This type of anlaysis would not be definitive with MPC-11 cells because a portion of the first exon (including the two normal c-*myc* promoters) remains on the reciprocal translocation product in this tumor (Stanton *et al.*, 1984), which is not the case in J558L (Gerondakis *et al.*, 1984).

DH sites in c-*myc* were then mapped in J558L and MPC-11 (see Figure 3). When the DNAs were digested with *Bam*HI and probed with c-*myc* 5′-flanking DNA, no DH sites were found in J558L or MPC-11 on the normal gene or on the reciprocal

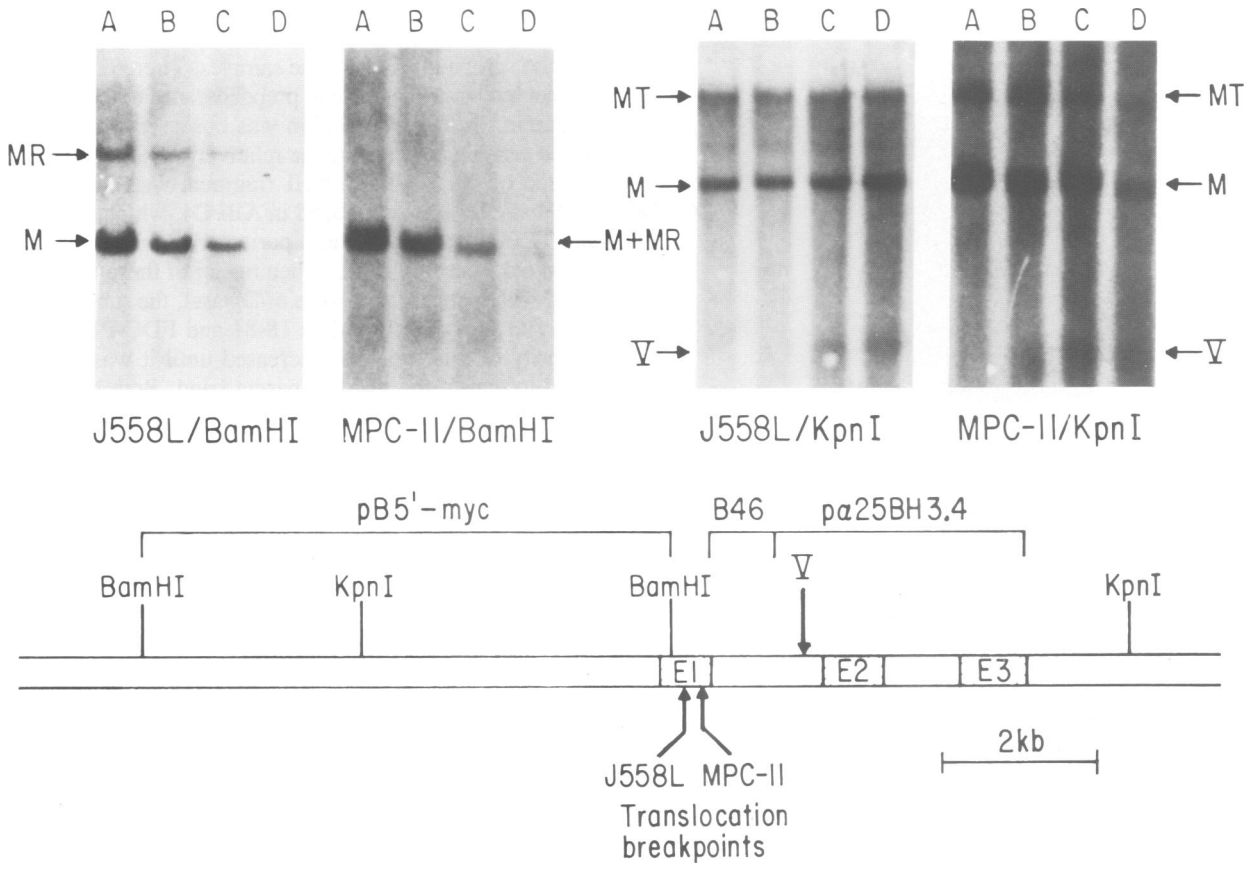

Fig. 3. DNaseI hypersensitivity in cells containing translocated, truncated c-*myc* genes. DNAs were prepared from DNaseI-treated nuclei and digested with *Bam*HI or *Kpn*I as indicated. *Bam*HI samples were probed with pB5′-myc; *Kpn*I digests were probed with pα25BH3.4 (shown) or B46 (data not shown). The translocation breakpoints within exon 1 are shown on the map. M, normal c-*myc* allele; MR, c-*myc* reciprocal; MT, translocated c-*myc*; V, sub-band generated by cleavage at DH site V. M and MR are distinguishable in J558L/*Bam*HI because of a deletion which occurred upstream of the breakpoint in this tumor as a consequence of translocation (Gerondakis *et al.*, 1984). The levels of DNaseI used were (units/2 × 10^7 nuclei): **lane A**, 0; **lane B**, 4.0; **lane C**, 8.0; **lane D**, 16 (*Bam*HI samples); **lane A**, 0; **lane B**, 2.0; **lane C**, 4.0; **lane D**, 8.0 (*Kpn*I samples). E1, E2 and E3 represent the normal gene's three exons. The breakpoints of the truncated c-*myc* genes in these two tumors are indicated.

translocation product. The same samples were digested with *Kpn*I and probed with DNA from the coding region. This probe detected only one DH site, which corresponded to site V in Figure 1. When both filters were probed with DNA upstream of DH site V (see B46, Figure 3), a different sub-band was detected (data not shown). This was not detectable with pα25BH3.4, perhaps because DH site V lies extremely close to the upstream limit of the probe. This new band was intermediate in size between the MT and M *Kpn*I bands in Figure 3, indicating that DH site V must exist on the larger, translocated c-*myc* *Kpn*I fragment. No sub-band lower than the germ line fragment was present. Thus, the translocated c-*myc* genes of J558L and MPC-11 have only one DH site (which lies near the cryptic promoters used in these tumors), whereas the normal genes in the same cells are devoid of DH sites.

Hypersensitive site I is detectable in the untranslocated c-myc in a Burkitt lymphoma

Our observation that DH site I was absent from transcriptionally silent c-*myc* was inconsistent with a previously published study (Siebenlist *et al.*, 1984) in which it was proposed that this site was associated with negative regulation of transcription in a Burkitt lymphoma. We decided, therefore, to map regions of DNaseI hypersensitivity on the normal c-*myc* allele of a Burkitt lymphoma, AW Ramos (Wiman *et al.*, 1984); the results are shown in Figure 4. Because the germ line and translocated c-*myc* genes in this cell line reside on *Sst*I fragments of greatly different sizes and because the translocation breakpoint occurs downstream of DH site I, we were able to show unambiguously that the sub-band designated I in Figure 4 arose from the untranslocated allele. No other DH sites were found to be associated with this copy of c-*myc*, a result consistent with that of a previous study of the same tumor (Dyson and Rabbitts, 1985).

Hypersensitive site I may distinguish translocated and normal c-myc in murine cell lines

To test the possibility that DH site I exists differentially in normal and translocated (but unrearranged) c-*myc*, we have mapped this site in several cell lines. Typical results are shown in Figure 5. The position of DH site I was confirmed by blotting analysis of the same DNAs following restriction with *Sst*I. By this criterion, DH site I was positioned upstream of and ~100 bp from the *Sst*I site as shown. FDC-P1 and 18-81 produce normal-sized mRNA from unrearranged c-*myc* alleles and two normal copies of chromosome 15. When nuclei of these cells are treated with increasing amounts of DNaseI, the 7.4-kb parent band is completely degraded, while the sub-band generated by cleavage at DH site I grows more intense. This suggests that site I is present on both c-*myc* alleles in these cells. This result was not apparent using the downstream probe for the *Kpn*I digests shown in Figure

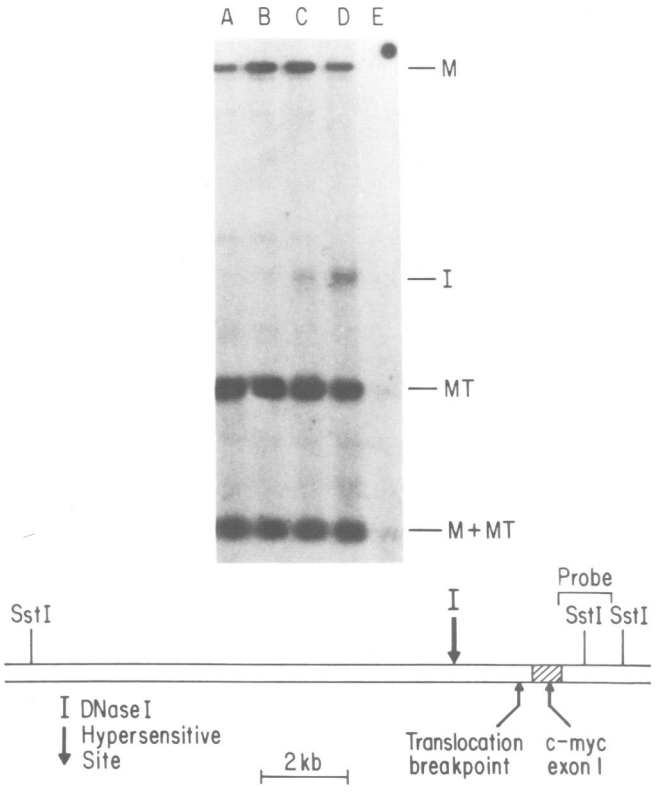

Fig. 4. Presence of DH site I on the normal c-*myc* allele in a Burkitt lymphoma. DNAs from DNaseI-treated nuclei of AW Ramos cells were digested with *Sst*I and analyzed by Southern blotting. The probe used was a 670-bp *Pvu*II/*Xba*I fragment from the 3′-portion of exon 1 and the 5′-portion of intron 1 of human c-*myc* (Siebenlist *et al.*, 1984). M, normal c-*myc* allele; MT, translocated c-*myc*; M + MT, downstream *Sst*I fragment arising from both copies of c-*myc*; I, sub-band arising from cleavage of M at DH site I. The levels of DNaseI used were (units/2 × 10^7 nuclei): **lane A**, 0; **lane B**, 2.0; **lane C**, 4.0; **lane D**, 8.0; **lane E**, 16.

1. The sub-band designated 'I' in *Kpn*I digests represents a partial DNase digestion product which, in turn, gives rise to the smaller sub-bands in the same samples. Therefore, this fragment did not tend to accumulate in preponderance to the residual parent fragment. Such accumulation was observed, however, when a probe capable of detecting the relatively DNase-stable, upstream portion of the 7.4-kb *Bam*HI fragment was employed.

DH site I was also mapped in ABPC4, which has a rcpt(6;15) and TEPC1198, which has a pericentric inversion of a Robertsonian 6;15 chromosome. When nuclei of these cells were incubated with increasing amounts of DNaseI, the sub-band associated with site I appeared. Unlike 18-81 and FDC-P1, however, the intensity of this sub-band increased until it was approximately equal to that of the residual parent band. Both bands were uniformly and completely degraded by higher amounts of DNaseI. Thus, it appears that only one copy of c-*myc* in each cell line has DH site I. DNaseI analysis of chromatin from several other cell lines yielded data consistent with that shown in Figure 5. Because we have previously demonstrated that an untranscribed c-*myc* gene lacks DH site I (see Figure 3), it seems reasonable to extrapolate this observation to other cell lines. Assuming that the translocated allele is transcriptionally active in plasma cell tumors while the normal c-*myc* gene is silent, we assembled the information shown in Table I.

Figure 5 also shows the result of similar analysis of 54c12, an Abelson murine leukemia virus-transformed fibroblast line which contains an amplified c-*myc* locus. In these cells we were able to detect the donwstream portion of the *Bam*HI fragment which results from cleavage at site I. This suggests that site I is of relatively greater hypersensitivity than the other sites which can be detected in these cells (see also Figure 1).

Mouse and human DNAs have regions of highly homolgous sequence near hypersensitive site I

The DNA sequence near DH site I in mouse was determined and compared with the corresponding region of the human gen-

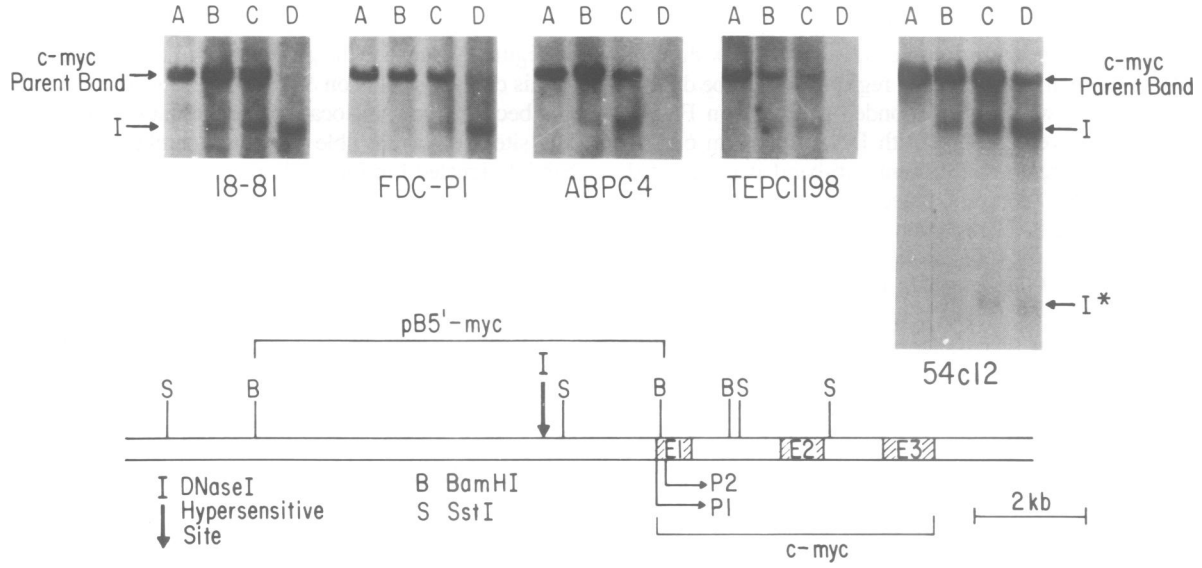

Fig. 5. DH site I in various murine cell lines. DNAs from DNaseI-treated nuclei were digested with *Bam*HI and subjected to Southern blot analysis; pB5′-myc was the probe. 18-81, ABPC4 and 54c12 are described in Figure 1. FDC-P1 is an IL-3 dependent myeloid cell line (Dexter *et al.*, 1980) and TEPC1198 is a plasma cell tumor containing a pericentric inversion of a Robertsonian 6;15 chromosome (Wiener *et al.*, 1984). The c-*myc* parent band is the 7.4-kb fragment corresponding to the probe region. I represents the sub-band generated by cleavage of chromatin at hypersensitive site I; the band designated I* in 54c12 arises from the region between site I and the downstream *Bam*HI site in c-*myc* exon 1. The position of site I was confirmed in *Sst*I digests with the same probe (data not shown). The levels of DNaseI used were (units/2 × 10^7 nuclei): **lane A**, 0; **lane B**, 2.0; **lane C**, 4.0; **lane D**, 8.0 (18-81, FDC-P1 and 54c12); **lane A**, 0; **lane B**, 4.0; **lane C**, 8.0; **lane D**, 16 (ABPC4 and TEPC1198). The c-*myc* exons and transcriptional start sites are designated as in Figure 1.

ome (Siebenlist *et al.*, 1984). The results of a computer-assisted analysis are presented in Figure 6. A similar analysis of mouse and human DNAs immediately downstream of this region revealed a baseline of ~50–60% homology with clusters of >80% homology near DH sites II_1, II_2, III_1 and III_2 (Corcoran *et al.*, 1985). Our results for the region near DH site I are consistent with these observations. The overall homology in the region tested was ~58%, while five fairly lengthy and numerous shorter runs of nucleotides were >90% homologous. The estimated location of DH site I mapped in human DNA (Siebenlist *et al.*, 1984) is consistent with our analysis of murine chromatin, which suggests that DH site I lies immediately upstream of the *Sst*I site as shown in Figures 5 and 6. Inspection of the five boxed regions of homology in Figure 6 shows that the distances from these sequences to the c-*myc* promoters is remarkably well conserved despite the presence of relatively non-homologous DNA in the intervening 1.5 to 2.0 kb.

Table I. Proposed presence of DH site I on c-*myc* alleles in various cell lines

Cell line	Contexts of c-*myc* alleles[a]	Presence of site I
J558L[b]	N/T(R)	−/−
MPC-11[b]	N/T(R)	−/−
NIH3T3	N/N	+/+
18-81	N/N	+/+
EA3-17	N/N	+/+
FDC-P1	N/N	+/+
C6T TEPC1156	N/T	−/+
CAK TEPC1198	N/T	−/+
ABPC4	N/T	−/+
ABPC20	N/T	−/+

[a]N, normal allele; T, translocated allele; T(R), translocated and rearranged (broken) allele.
[b]Region of DNA which would contain site I is on the reciprocal chromosome.

Discussion

DNaseI hypersensitive sites in intact and truncated c-myc

Table II summarizes the results of a fairly extensive survey of DH sites associated with murine c-*myc* loci in various cell types and genomic contexts. In location, but not necessarily occurrence, sites I, II, III and V correspond to those mapped previously in human c-*myc* (Siebenlist *et al.*, 1984; Dyson and Rabbitts, 1985). Site IV, which was detectable only in a cell line which contained a c-*myc* amplification, maps in the 5′ portion of intron 1, similar to a site described elsewhere (Tuan and London, 1984). DH site IV, if it exists in the cell lines which lack c-*myc* amplification, may be very weak and therefore undetectable. We cannot exclude the possibility, however, that this site is associated uniquely with amplified c-*myc*.

DH sites II and III, each of which can be further resolved into two components (Siebenlist *et al.*, 1984), may have more readily apparent functional significance with respect to c-*myc* transcription. These sites lie within a region where the murine and human c-*myc* loci share substantial sequence homology (Bernard *et al.*, 1983; Siebenlist *et al.*, 1984; Corcoran *et al.*, 1985). Furthermore, DH site II_1 has been shown to be near a region which binds purified nuclear factor 1 (Siebenlist *et al.*, 1984), while sites III_1

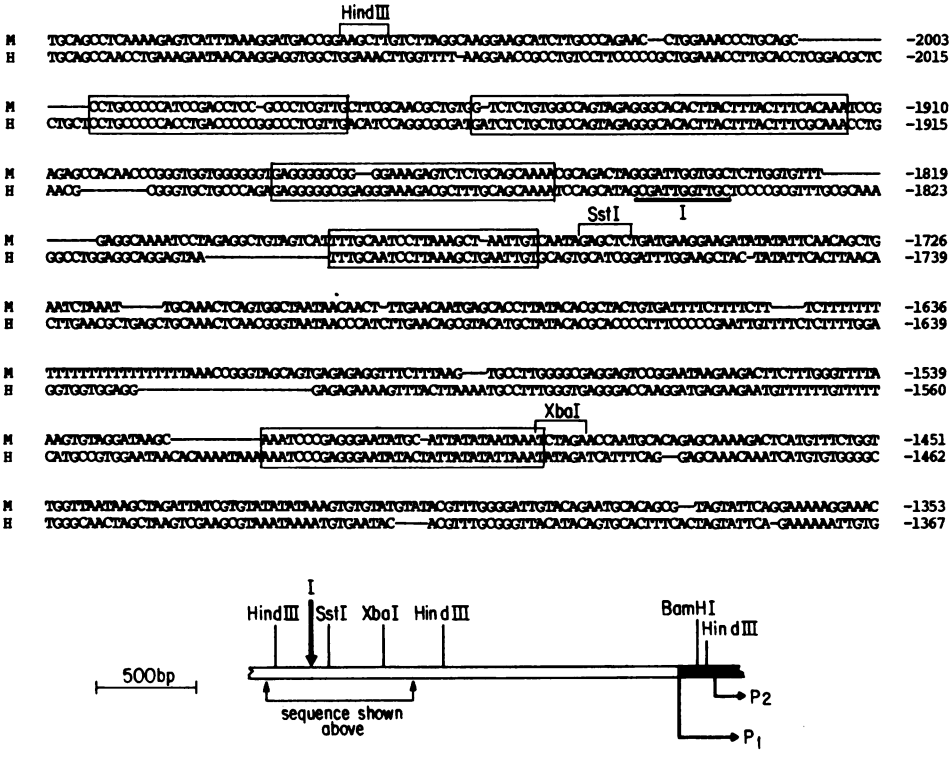

Fig. 6. Nucleotide sequence comparison of mouse and human DNA near hypersensitive site I. The mouse sequence (M) was determined and aligned with the corresponding region of human (H) c-*myc* flanking DNA (positions −2278 to −1532 by the numbering system of Siebenlist *et al.*, 1984). Maximum homology was obtained by computer analysis, resulting in occasional gaps in one sequence with respect to the other. Five regions of DNA >20 nucleotides in length and >90% homologous were found and are indicated by boxes. Both sequences are numbered with respect to the P1 initiation site (+1). A map illustrating the position of this sequence with respect to the c-*myc* promoters and exon 1 is shown below. The region designated 'I' on the map shows the location of DH site I as determined by Southern blotting in our experiments. The DNA sequence labeled 'I' is the region previously identified as the approximate center of DH site I in human DNA (Siebenlist *et al.*, 1984).

Table II. Summary of DH sites near c-*myc* in murine cell lines

Cell line	Description[a]	Hypersensitive site				
		I	II	III	IV	V
NIH3T3	Fibroblast	+	+	+	−	+
54c12	A-MuLV fibroblast	+	+	+	+	+
18-81	A-MuLV pre-B lymphoid	+	+	+	−	+
EA3-17	Pre-B lymphoid	+	+	+	−	+
FDC-P1	Myeloid	+	+	+	−	+
MPC-11	PCT,rcpt(12;15)	−	−	−	−	+[b]
J558L	PCT,rcpt(12;15)	−	−	−	−	+[b]
C6T TEPC1156	PCT,rcpt(12;15)	+	+	+	−	+
CAK TEPC1198	PCT,inv(6;15)	+	+	+	−	+
ABPC4	PCT,rcpt(6;15)	+	+	+	−	+
ABPC20	PCT,rcpt(6;15)	+	+	+	−	+

[a]A-MuLV, Abelson murine leukemia virus; PCT, plasma cell tumor; rcpt, reciprocal chromosome translocation; inv, pericentric inversion.
[b]Site V is present only on the translocated allele.

and III_2 map near the two promoters normally employed by unbroken genes (Bernard *et al.*, 1983; Yang *et al.*, 1985; Siebenlist *et al.*, 1984). We presume, therefore, that these sites are directly linked with c-*myc* transcriptional activity.

The presence of site V within c-*myc* in all mouse cells studied may have functional significance for those tumors in which c-*myc* has been truncated by chromosome translocation (e.g., J558L and MPC-11). This DH site maps in a portion of c-*myc* intron 1 which has been shown to contain the cryptic promoters employed by such broken genes (Stanton *et al.*, 1983; Keath *et al.*, 1984). Our results suggest that the chromatin structure of this region is unaltered by translocation, although the transcriptional start sites which it contains function only when the normal, upstream promoters are removed (Stanton *et al.*, 1983; Marcu *et al.*, 1984a, 1984b). DH site V may identify a transcriptional control element which is responsible for the activity of the intron promoters. Furthermore, these promoters may also be dependent upon *cis*-acting immunoglobulin regulatory sequences lying upstream of the truncated c-*myc*. DH site V was found in human c-*myc* as well, but it was fairly weak and not present consistently (Siebenlist *et al.*, 1984). Interestingly, gene breakage and use of intron promoters is relatively rare in human tumors, whereas it was found to be fairly common in murine c-*myc* translocations (Klein, 1984; Perry, 1983). In a similar sense, we have found that sites II_1 and II_2 are sometimes undetectable in the mouse cells tested, while the previous studies of human c-*myc* (Siebenlist *et al.*, 1984; Dyson and Rabbits, 1985) consistently revealed these sites. In view of the high degree of sequence homology between mouse and human DNAs in these regions (Corcoran *et al.*, 1985) we consider it likely that the discrepancies mentioned above may be cell type- or differentiation-specific as opposed to fundamental, species-related differences in c-*myc* transcriptional control.

Possible significance of hypersensitive site I

The major DH site we found, site I, maps ~2 kb upstream of c-*myc*. Although this site is at a relatively great distance from the c-*myc* transcription unit and any of the gene's defined regulatory elements, the sequence homology between this region in mouse and human DNA (Battey *et al.*, 1983; Siebenlist *et al.*, 1984) suggests that it may have some functional significance. In the present study, mapping of DH site I in murine chromatin by Southern blotting and direct comparison of DNA sequence support this conclusion. Several blocks of clustered, highly conserved regions of DNA (~20–40 nucleotides) were found in the vicinity of DH site I, interspersed with shorter stretches of homologous and non-homologous sequences and at a conserved distance from c-*myc*. One obvious conclusion is that the highly conserved regions may represent recognition sequences for specific factors which may bind to the DNA (giving rise to the observed nuclease hypersensitivity nearby) and thereby exert a regulatory influence on c-*myc*.

DH site I has been proposed to be linked to repression of transcriptional activity of human c-*myc*, although it was found on both normal and translocated c-*myc* alleles (Siebenlist *et al.*, 1984; Dyson and Rabbitts, 1985). Our results with a number of murine cell lines do not support this conclusion. First, genes which are not transcribed (i.e., the normal c-*myc* alleles of J558L and MPC-11) lack site I as well as all other DH sites. Second, cells which possess two contextually normal, transcriptionally active c-*myc* genes (NIH3T3, 18-81, FDC-P1, EA3-17) appear to have site I on both alleles. Conversely, cell lines containing translocated but unrearranged c-*myc* (ABPC4, ABPC20, TEPC1156, TEPC1198) appear to have DH site I on only one allele (see Figure 5 and Table I). These data are more consistent with association of site I with expression rather than repression of c-*myc*.

While DH site I may not be linked to repression, we do not have evidence that it functions necessarily to activate transcription. In a result consistent with Siebenlist *et al.* (1984) and Dyson and Rabbits (1985), we found site I on the untranslocated (untranscribed) c-*myc* allele in a Burkitt lymphoma. In addition, this region of 5′-flanking DNA is frequently separated from c-*myc* by translocation. It seems unlikely, therefore, that site I reflects the presence of an essential enhancer-like function. Perhaps the simplest way to reconcile these observations lies in the fact that DH sites may arise either as a consequence of DNA secondary structure (Weintraub, 1983) or of specific interaction of DNA with binding factors (Weisbrod, 1982; Emerson and Felsenfeld, 1984; Zaret and Yamamoto, 1984). It is possible that the presence of DH site I is indicative of a general state of transcriptional competence of c-*myc*. In this sense, we could say that overall chromatin structure associated with the presence of this site may be necessary for transcription of a gene whose 5′-flanking region is in germ line configuration, but is not, of itself, sufficient to result in transcriptional activity. An extension of this may be that DH site I defines the upstream limit of DNaseI sensitivity of transcriptionally competent c-*myc* chromatin. It has been shown recently that several DH sites upstream of *Tetrahymena* rRNA genes coincide with repeated topoisomerase I recognition sequences (Bonven *et al.*, 1985). This enzyme has been proposed to have a role in maintaining a chromatin structure associated with transcriptional activity (Glikin *et al.*, 1984).

Chromosome translocations and c-myc activation in plasma cell tumors

Several alternative chromosome translocations involving c-*myc* are believed to result in its 'dysregulation' in malignancies of the B-cell lineage. Some of these disrupt putative regulatory elements immediately upstream of c-*myc* and often result in loss of the normal promoters, in addition to bringing possible *cis*-acting immunoglobulin regulatory sequences into the gene's proximity. Others, most notably the variant translocations, occur in a 4.5-kb cluster (the *pvt*-1 locus) at least 72 kb from c-*myc* (Cory *et al.*, 1985). Three tumors in the present study, ABPC4, ABPC20 and TEPC1198, fall into this class. The question of c-*myc* activation at a distance as a consequence of this translocation has been addressed and a long range *cis* effect on c-*myc*

chromatin by disruption of *pvt*-1 was one proposal (Cory *et al.*, 1985). The apparent absence of DH site I on one c-*myc* allele in each of these tumors would directly support the idea that their untranslocated c-*myc* are transcriptionally silent. Recently, it has been demonstrated that the relative rates of c-*myc* transcription in ABPC20 and 18-81 are virtually the same (Piechaczyk *et al.*, 1985) and this is also apparent for ABPC4 (Figure 2). This result is consistent with our observation that translocation within the *pvt*-1 locus in some plasma cell tumors yields a c-*myc* chromatin structure which is indistinguishable from that of a transcriptionally active, untranslocated gene.

Plasma cell tumors are malignancies of terminally differentiated immunoglobulin-secreting cells. Presumably, growth of these neoplastic cells is somehow related to constitutive expression of c-*myc*, a gene which also appears to play a role in normal cell division. Because the plasma cell (normal cellular counterpart of the plasma cell tumor) is fully differentiated and thought to be non-proliferative, c-*myc* may lie in a closed chromatin domain and may acquire transcriptional competence as a result of chromosome translocation. The transition from transcriptionally competent to inactive chromatin is believed to be gradual, involving a series of steps. It follows that the nature of a translocation needed to reactivate c-*myc* (i.e., those translocations which are growth-selected) may depend upon the precise state of chromatin structure associated with the stage of differentiation at which this event occurs.

Materials and methods

Cell lines

The cell lines used in these experiments have been described elsewhere: J558L (Oi *et al.*, 1983); MPC-11 (Laskov and Scharff, 1970); other plasma cell tumors (Ohno *et al.*, 1984; Wiener *et al.*, 1984); 18-81 (Alt *et al.*, 1981); EA3-17 (Palacios *et al.*, 1984); FDC-P1 (Dexter *et al.*, 1980). 54c12 is an A-MuLV transformed NIH3T3 cell line and was provided by N.Rosenberg.

Preparation of DNaseI-treated samples

Nuclei were prepared and treated with DNaseI (P-L) essentially as described (Siebenlist *et al.*, 1984) except that each DNaseI-treated sample consisted of $\sim 2 \times 10^7$ nuclei. Nuclei were lysed following incubation with DNaseI and DNA was purified by proteinase K digestion, multiple extractions with phenol:chloroform and ethanol precipitation.

Southern blotting and DNA probes

Genomic DNAs (10 μg per sample) were digested with the appropriate restriction enzyme, electrophoresed on 1% agarose gels and transferred to nitrocellulose filters (Southern, 1975). Conditions of hybridization to labeled DNA probes were as described (Harris *et al.*, 1982). The probes used were: pB5′-myc, a 7.4-kb *Bam*HI fragment of c-*myc* 5′-flanking DNA cloned in pBR322; pα25BH3.4 (Harris *et al.*, 1982); pBH1.1, a 1.1-kb *Bam*HI/*Hind*III fragment of c-*myc* 3′-flanking DNA cloned in pBR322; B46, a 700-bp *Bam*HI/*Bgl*II fragment containing the 5′-portion of mouse c-*myc* intron 1 DNA cloned in M13 mp8. The *Pvu*II/*Xba*I human c-*myc* fragment was provided by J.Battey.

Elongation of transcripts in isolated nuclei

Nuclei were purified and nascent transcripts elongated and labeled as described by Schibler *et al.* (1983). Spotting of DNA on nitrocellulose filters, pre-hybridization, hybridization and washing are described elsewhere (Piechaczyk *et al.*, 1984). The c-*myc* exon 1 probe was pR*s_{10} (Yang *et al.*, 1985) and the exon 2 and 3 probe was pMYC7.4 (Watt *et al.*, 1983).

DNA sequence analysis

DNA sequences were determined by the dideoxy chain termination method (Sanger, 1977) using subcloned fragments in bacteriophage M13 (Messing *et al.*, 1981). Computerized determination of DNA sequence homology was performed using the nucleic acid alignment program of D.J.Lipman and W.J.Wilbur, Mathematical Research Branch, N.I.A.D.D.K., National Institutes of Health, Bethesda, MD.

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