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Chromatin structure around the c-*myc* gene in Burkitt lymphomas with upstream and downstream translocation points

(oncogene)

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ABSTRACT **Burkitt lymphoma cells seem to have abnormal c-*myc* gene activity resulting from chromosomal translocation. We have examined the consequences of translocation on putative control sequences near to the c-*myc* gene by DNase I hypersensitivity mapping of chromatin. There is no detectable difference in the pattern of hypersensitivity (compared with the actively transcribed c-*myc* gene of lymphoblastoid cells) in Burkitt lymphoma cells where the translocation point occurs at a considerable distance upstream or downstream of c-*myc*. When the translocation occurs near the 5′ end of the c-*myc* gene, resulting in loss of hypersensitive sites, those that remain show the same sensitivity as in lymphoblastoid cell lines. We conclude that translocation has little general effect on the usual pattern of hypersensitive sites near to the c-*myc* gene but new sites can be observed in some cases in the immunoglobulin region near to the breakpoint. These may be sites normally involved in immunoglobulin gene transcription and may exert a subtle effect on the translocated c-*myc* gene.**

The c-*myc* proto-oncogene undergoes two types of genetic rearrangement in tumors—namely, chromosomal amplification (1, 2) and translocation (3). In the latter case, in the human B-cell tumor, Burkitt lymphoma (BL), there is complete correlation between the presence of one of three types of chromosomal translocation and the breakpoint of 8q24, which is the position of the c-*myc* gene (4–6). Studies on the steady-state levels of *myc* mRNA in BL cells have shown that most usually translocation has no radical effect compared to lymphoblastoid cells (LCLs) (7, 8). It is probable therefore that the translocation affects the expression pattern of c-*myc* in such a way that it becomes unregulated and continually expressed but in essentially normal quantities. We sought to obtain information on how such an effect could be mediated in a variety of BL cells by analyzing the sequences potentially involved in control of *myc* gene activity as exemplified by chromatin sites that are hypersensitive to digestion with DNase I. Such sites represent important sequences near to active genes and have recently been demonstrated near to the c-*myc* gene (9, 10), although the results have been conflicting regarding the number and location of these sites. Furthermore, only one BL cell has so far been examined and it is one in which the major immunoglobulin enhancer was near to the translocated c-*myc* gene (10).

We have now studied four different BL cell lines and compared these with two LCLs (one nontumorigenic and one tumorigenic). Five major sites of DNase I hypersensitivity occur near actively transcribed c-*myc* genes in both BLs and LCLs but no obvious changes occur in this pattern of sites after the translocation (except where sites are removed by translocation). However, new hypersensitive sites can occur in the immunoglobulin region near the breakpoint, which could play a role in controlling c-*myc* activity.

MATERIALS AND METHODS

DNase I Digestion and DNA Analysis. Preparation of DNase I-treated nuclei was essentially as described (11, 12). Between 2 and 5 × 10^8 cells in logarithmic phase were sedimented, washed, and swollen in 10 mM Tris·HCl, 7.4/10 mM NaCl/5 mM $MgCl_2$ for 30 min at 0°C. The cells were then broken by homogenization after addition of Nonidet P-40 to 1.75%. Nuclei were collected, resuspended in 10 mM Tris·HCl, 7.4/10 mM NaCl/3 mM $MgCl_2$, and digestion was carried out at 37°C for 5 min with 0.1–50 μg of pancreatic DNase I (Sigma) per ml. Reactions were terminated by addition of EDTA to 2 mM followed by DNA extraction (13). After restriction enzyme digestion, the DNA was fractionated on agarose gels, transferred to cellulose nitrate (14), and hybridized with nick-translated probes as described (15–17).

DNA Sequencing. DNA sequencing was carried out by using dideoxy chain-termination procedures in M13 single-stranded DNA vectors (18–20).

RESULTS

DNase I Sensitivity of the Normal c-*myc* Gene. The c-*myc* gene is transcribed in a wide variety of actively growing cell types. BL and LCL lines transcribe c-*myc* genes at approximately equivalent levels (7, 8) but their usage of the two promoters (P1 and P2) of the gene is variable. For example, growing cultures of Raji cells express *myc* RNA predominantly from P1 (7), HMy2, Daudi, and Ramos cells express *myc* RNA from P1 and P2 about equally, and DHLCL express *myc* RNA predominantly from P2 (unpublished data). Table 1 shows a list of cell lines used in this study and the translocations that they contain. This differing promoter usage is intriguing and indicated the possibility that chromatin structure, which reflects gene activity, may be detectably different around the c-*myc* gene in these different types of cell line.

We initially characterized DNase I-hypersensitive sites upstream of the active c-*myc* gene in the DHLCL line. This line is an Epstein–Barr virus-transformed peripheral blood lymphocyte cell that does not contain a chromosomal translocation (unpublished data). The results of DNase I mapping are shown in Fig. 1. An ethidium bromide-stained gel that illustrates the effect of increasing DNase I concentration on the overall molecular weight of the nuclear DNA is shown in Fig. 1*A*. This same DNA was digested with the restriction enzyme *Bgl* II [which cleaves the *myc* gene just 3′ to exon 2 and 2.9 kilobases (kb) upstream of exon 1 to yield a fragment

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Abbreviations: LCL, lymphoblastoid cell; BL, Burkitt lymphoma; kb, kilobase(s).

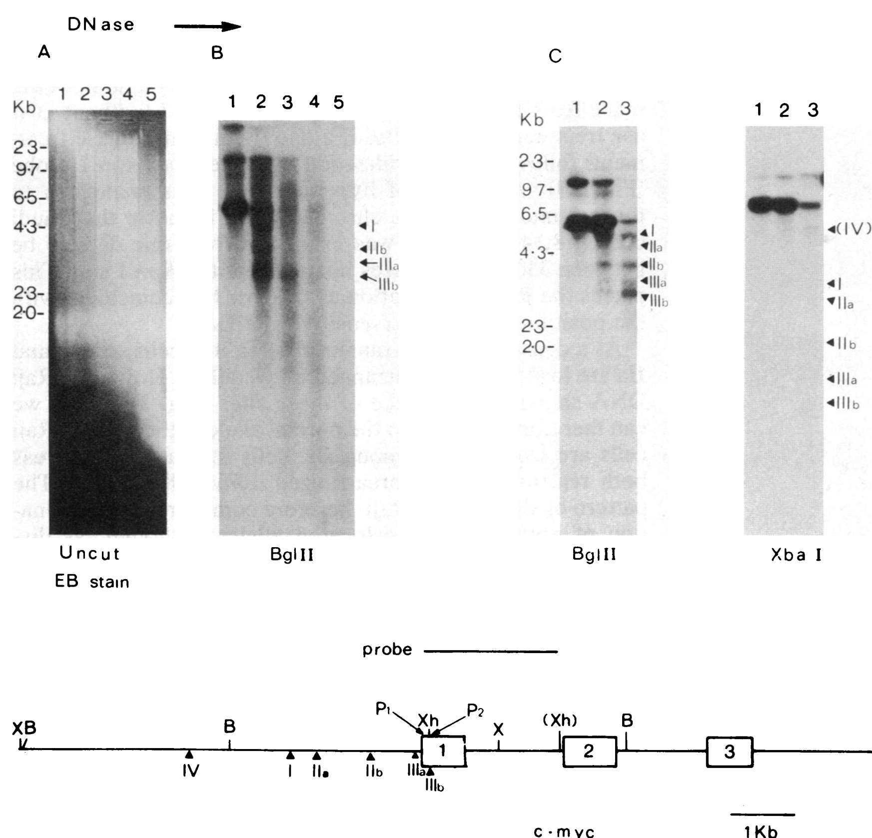

FIG. 1. DNase I-hypersensitive sites near the c-*myc* gene in a human LCL line. (*A*) Ethidium bromide (EB)-stained 1% agarose gel of DHLCL DNA prepared from nuclei treated with various amounts of DNase I. Lane 1, uncut; lane 2, 8 μg/ml; lane 3, 20 μg/ml; lane 4, 25 μg/ml; lane 5, 30 μg/ml. Sizes in kilobases are from coelectrophoresis of λ phage DNA cut with *Hin*dIII. (*B*) Ten-microgram aliquots of the same DNA samples as in *A* were digested to completion with *Bgl* II, fractionated on a 0.8% agarose gel, and transferred to cellulose nitrate (14). The filter was hybridized with the *myc* exon 1 probe [pUCLYXh16 (17)] indicated at the bottom of the figure. After washing, the filter was autoradiographed. The positions of hypersensitive sites in the chromatin are indicated. (*C*) DNA samples from DHLCL cells from DNase I-digested and undigested nuclei were cut with *Bgl* II or *Xba* I, fractionated on a 1.2% agarose gel, blotted, and hybridized to the exon 1 probe. Lane 1, uncut; lane 2, 0.6 μg of DNase I per ml; lane 3, 1.8 μg of DNase I per ml. The bottom panel shows a map of the human c-*myc* gene together with locations of the two c-*myc* promoters (P1 and P2) and the DNase I-hypersentive sites (I–IV). Note that the location of sites IIIa and IIIb approximate to the two c-*myc* promoters and the positions marked with arrowheads are only an indication of the location. X = *Xba* I; B = *Bgl* II; Xh = *Xho* I.

of 5.9 kb] followed by gel fractionation, transfer to cellulose nitrate, and hybridization to the exon 1 probe (indicated at the bottom of Fig. 1). Fig. 1*B*, lane 1, shows the main hybridizing band corresponding to the *myc* gene at 5.9 kb in *Bgl* II-digested DNA not treated with DNase. (More weakly hybridizing higher molecular weight bands are also visible, which probably represent cross-hybridizing sequences, since washing the filters at higher stringency removes these components.) In Fig. 1*B*, lanes 2–5, following DNase I digestion of the nuclei, we observed the appearance of new bands representing hypersensitive sites upstream of c-*myc*. Fig. 1*B* clearly distinguishes four such sites, designated I, IIb, IIIa, and IIIb. A fifth major site, IIa, was revealed in a different fractionation of *Bgl* II-digested DNA (Fig. 1*C*), and yet a further site, which is variable in its appearance, designated IV, could be detected when enzymes, such as *Xba* I, were used (see Fig. 1*C*). Estimation of the size of these fragments from several different experiments allows the hypersensitive sites to be positioned relative to the c-*myc* gene, as shown at the bottom of Fig. 1. These positions were roughly in agreement with those shown previously (10), sites IIIa and IIIb corresponding to the P1 and P2 promoters of the *myc* gene. All of the sites appear to have approximately the same sensitivity to DNase I as sites found in an expressed part of the Epstein–Barr virus gene independently investigated (unpublished data) and presumably represent functionally important regions of the chromatin around c-*myc*.

Table 1. Cell lines used in this study and the translocations they contain

Cell line	Translocation	Breakpoint relative to c-*myc*
BL		
Raji	8;14	Upstream
Ramos	8;14	Upstream
Daudi	8;14	Upstream
JI	2;8	Downstream
Lymphoblastoid		
DHLCL	—	—
HMy2	—	—

DNase I-Hypersensitive Sites in BL Cells. The possibility that chromosomal translocation affects the pattern of *myc* hypersensitive sites was investigated in several BL cell lines. We selected cells in which the translocation point was at least 20 kb upstream of c-*myc* [Daudi, t(8;14) (17, 23)] or at least 12 kb downstream of c-*myc* [JI, t(2;8) (17, 24)] or where the breakpoint was close to but not within c-*myc* [Raji (7) and Ramos, t(8;14) (23)] (see Table 1). In addition, the latter two cell lines do not retain the immunoglobulin enhancer on the $14q^+$ chromosome. The DNase I sensitivity of chromatin from the various BL cells was compared with the DHLCL (Fig. 2) by using a probe from the region covering exon 2 of c-*myc*. The pattern of sites found in DHLCL, JI, and Daudi chromatin is essentially similar (see also Fig. 3*B*).

In Raji and Ramos cells the translocation point occurs near exon 1 on c-*myc* (7, 23) at the positions indicated at the bottom of Fig. 2. The approximate position of the Raji breakpoint has been shown previously to be upstream of the *Kpn* I site shown in the restriction map (24). A site for *Cla* I (boxed in the sequence shown in Fig. 3) occurs just 5′ of the *Kpn* I site and we utilized the former to allow DNA sequencing of the region covering the translocation point. This sequence is shown in Fig. 3*A*, in which the Raji translocated and normal sequences upstream of the *Cla* I site are compared. The sequences are identical for about 145 nucleotides from the *Cla* I site and then they diverge. The translocated gene sequence after this point has the characteristic pattern of repeats (in reverse orientation) seen in the immunoglobulin switch region of the C_μ gene 5′ A-G-C-T-C-A-G-C-C-C 3′ / 3′ T-C-G-A-G-T-C-G-G-G 5′ and corresponds to the start of the amalgamated S_μ/S_γ region described previously (24). The Raji translocation point is therefore established at 339 bases from the *Kpn* I site shown

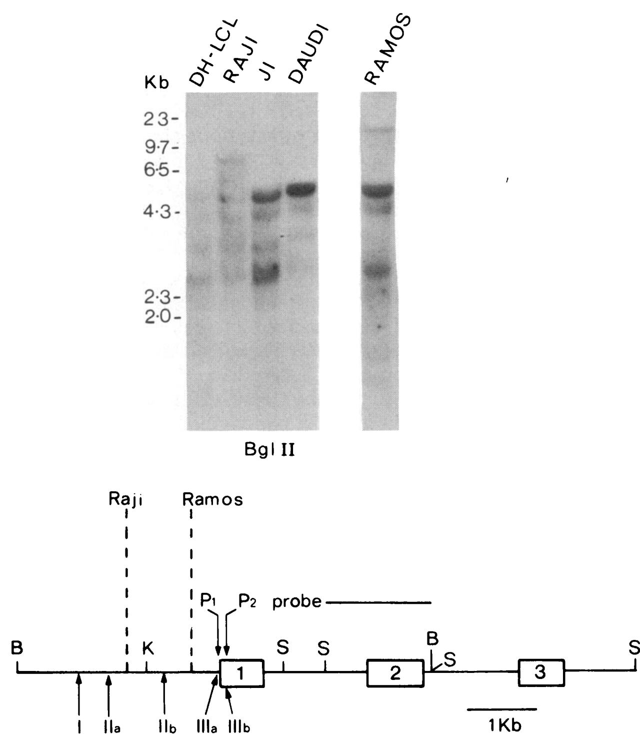

FIG. 2. Comparison of DNase I-hypersensitive sites in various BL cell lines. Nuclei from the cell lines were treated with DNase I prior to digestion with *Bgl* II; the required amount of DNase (which varied between cell lines) was titrated in pilot experiments (the amounts used were 8 μg/ml for DHLCL, 1.5 μg/ml for Raji, 1.5 μg/ml for JI, 3 μg/ml for Daudi, and 0.5 μg/ml for Ramos). The digested DNA was fractionated and hybridized as in Fig. 1 except that an exon 2 probe (M13J171.5 Sac) (7) was used (indicated in the lower panel). Sizes in kilobases correspond to λ phage DNA cut with *Hin*dIII. The lower panel shows the c-*myc* gene restriction map and the approximate location of the translocation points in Raji and Ramos DNA. B = *Bgl* II; K = *Kpn* I; S = *Sac* I.

in the map at the bottom of Fig. 2. The relative positions of the Raji breakpoint, hypersensitive site IIa, and the *Kpn* I site were determined by digesting genomic DNA (in this case made from Daudi cells) with *Bgl* II and *Kpn* I (without DNase treatment) or with *Bgl* II alone (with prior DNase treatment) followed by hybridization with the *myc* exon 1 probe (Fig. 3*B*). A pattern of hypersensitive sites analogous to those found in DHLCL chromatin was found in the Daudi cells [t(8;14)]. Site IIa was estimated from this data to be between 330 and 350 bases upstream of the *Kpn* I site. This places the Raji translocation as more or less coincident with the position of the hypersensitive site IIa.

As a consequence of translocation in Raji cells, sites I and IIa are lost from the rearranged c-*myc* allele. However, Raji DNA shows the presence of these sites (Fig. 2), which we can therefore attribute to the normal allele in these cells. Raji cells are exceptional among BL cells in that they express both rearranged and unrearranged c-*myc* alleles (17). The pattern of sites seen in Raji therefore comes from a combination of normal and translocated alleles (although, as discussed below, this probe detects predominantly the normal allele). In Ramos cells the situation differs because the breakpoint removes sites I, IIa, and IIb from the rearranged allele (23). Fig. 2 shows, however, that site I, but not sites IIa and IIb, exists in Ramos chromatin; therefore, site I must occur in the chromatin of the normal, presumably inactive, c-*myc* gene. Sites IIIa and IIIb are present in Ramos chromatin but we cannot determine from these data whether the inactive allele possesses these sites.

DHLCL, used in this study, is an Epstein–Barr virus-transformed peripheral blood lymphocyte cell line and is nontumorigenic (unpublished data). We have compared the DNase I sensitivity of this to a tumorigenic LCL (HMy2) and the results are shown in Fig. 4. When the exon 1 fragment was used as a probe (designated normal *myc* probe and shown in the lefthand panel), we found the pattern of sites in the two types of LCL to be indistinguishable, each exhibiting

A

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Raji    TAGACCGCCA GACAGCCCAG CCAGCCCAGC TCAGCCCAGC CCAGTTCAGC TCAGCCCAGC
                                                                       60
Normal  ATTTCAGGGA GCAAACAAAT CATGTGTGGG GCTGGGCAAC TAGCTGAGTC GAACCGTAAA
R       CCTGCCCAGC TCAGCCCGCC TTAGTGCAGC CAAGCCCAGG TCAACTCAGT ATTCAGAAAA
                                                                       120
N       TAAAATGTGA ATACACGTTT GCGGGTTACA TACAGTGCAC TTTCACTAGT ATTCAGAAAA
R       AATTGTGAGT CAGTGAACTA GGAAATTAAT GCCTGGAAGG CAGCCAAATT TTAATTAGCT
                                                                       180
N       AATTGTGAGT CAGTGAACTA GGAAATTAAT GCCTGGAAGG CAGCCAAATT TTAATTAGCT
R       CAAGACTCCC CCCCCCCCC- AAAAAAAGGC ACGGAAGTAA TACTCCTCTC CTCTTCTTTG
                                                                       240
N       CAAGACTCCC CCCCCCCCCC AAAAAAAGGC ACGGAAGTAA TACTCCTCTC CTCTTCTTTG
R       ATCAGAATCG AT

N       ATCAGAATCG AT
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B Daudi

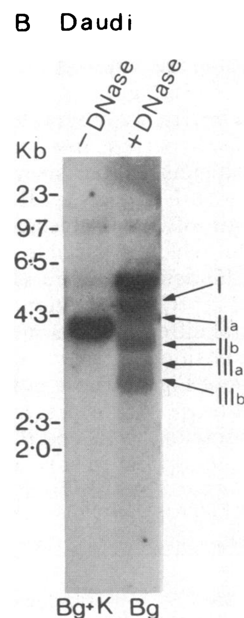

FIG. 3. Sequence of the chromosomal translocation point near the c-*myc* gene in Raji cells. (*A*) The sequence of normal and translocated c-*myc* gene from Raji. *Cla* I- plus *Hin*dIII-digested clones [λRB19 and λRB6 (7)] containing the two types of Raji c-*myc* gene were ligated in M13mp9 and sequenced by the dideoxy chain-termination procedure (20). The box shows the *Cla* I site and the upper sequence in each line comes from the translocated gene (designated Raji) and the lower sequence comes from the unrearranged gene (normal). The region of similarity is indicated by the line between the sequences. (*B*) Filter hybridization of Daudi DNA with the exon 1 probe. Daudi DNA was either digested with *Bgl* II (Bg) after pretreatment of nuclei with 3 μg of DNase per ml or digested with *Bgl* II plus *Kpn* I (Bg+K) without DNase pretreatment, fractionated on a 1% agarose gel, and hybridized, as in Fig. 2, with the *myc* exon 1 probe. Positions of bands resulting from DNase I sites are indicated and sizes in kilobases are estimated from the coelectrophoresis of λ phage DNA cut with *Hin*dIII.

the five sites discussed, even though there is a significant difference in the ratio of P1 and P2 promoter usage in these two cell lines. (The band labeled N in Fig. 4 corresponds to the *Bgl* II restriction fragment containing the *myc* gene present in each cell line prior to DNase I digestion.)

New DNase I-Hypersensitive Sites in Translocated c-*myc* of Raji. Both Raji and Ramos DNA exhibit new c-*myc* restriction fragments corresponding to the c-*myc* gene of the translocation; these appear as bands with higher molecular weights than the 5.9-kb unrearranged *Bgl* II bands in the appropriate lanes of Fig. 2. In Raji DNA, in addition to the normal pattern of hypersensitive sites, there is an indication of new sites in the translocated c-*myc* allele and which, by virtue of their size, must come from the immunoglobulin segment of the DNA. We have attempted to clarify the existence of immunoglobulin-associated hypersensitive sites by using a probe for the Southern blot that might discriminate the rearranged from the normal Raji c-*myc* gene. The normal *myc* gene (labeled N in Fig. 4) predominates over the translocated allele in Raji when a normal exon 1 probe is used, and consequently the pattern of hypersensitivity due to the normal, active Raji gene is seen. When we used a probe from the Raji translocated allele that contains many deletions and mutations (24) (see Fig. 4), we could show that the translocated gene (T) hybridized more strongly than the normal gene (N) (righthand panel). (Daudi DNA cut with *Bgl* II is included in Fig. 4 as a reference for the normal *myc* allele.) When Raji nuclei were subjected to DNase I treatment prior to *Bgl* II digestion, we found that the Raji translocated *myc* probe detected at least two new hypersensitive sites (sizes around 7 kb), which must come from the immunoglobulin region of the translocated chromosome and might represent new sites near the translocation involved in control in this cell. The hypersensitive sites from the normal c-*myc* are faintly visible below the 5.9-kb *myc* band when using this probe.

DISCUSSION

Our results show that there are five major sites of DNase I hypersensitivity in the chromatin around the active c-*myc* gene in LCL and variably in BL cell lines. The locations of these sites are summarized in Fig. 5. The data are qualitatively consistent with previous results on a LCL and a BL cell (10), though differing from other data obtained with different types of cell (9). It would be of interest to ascertain whether or not such discrepancies reflect the differentiation states of the cell types used. The data on Ramos chromatin show that the inactive allele does not have site IIb, whereas active genes have all five sites, except when chromosomal translocation removes one or more of them. These sites presumably represent regions of the chromosome important for the functioning of the c-*myc* gene and one of these sites (equivalent to site IIa here) is known to bind nuclear factor 1 (10). The possibility that one or more of the other sites represents the location of binding of a feedback repressor molecule must be considered. It is known that the human c-*myc* protein has a nuclear localization (ref. 25 and unpublished data), and experiments are necessary to test the hypothesis that the c-*myc* protein itself binds to the upstream region of the c-*myc* gene.

It is intriguing that variable loss of DNase I-hypersensitive sites in chromatin can occur in BLs, without any obvious correlation to changes in RNA levels. For example, there are three sites left in translocated *myc* in Raji, two in Ramos, and, of course, all five in the untranslocated c-*myc* of LCLs. Further, it is interesting that the sites that seem to correlate

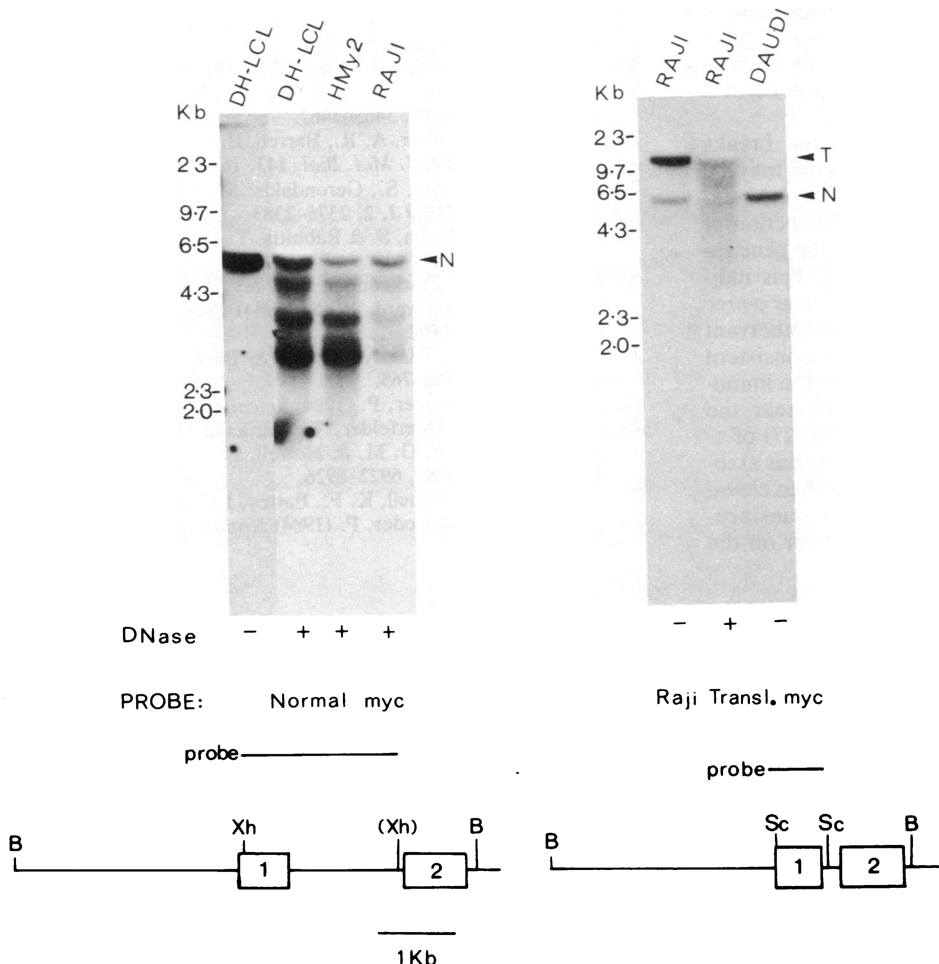

FIG. 4. Analysis of chromatin hypersensitive site near the translocation point in Raji cells. DNAs were fractionated on 1.0% agarose after digestion by *Bgl* II with (+) or without (−) prior treatment of nuclei with DNase I. The gels were blotted and hybridized as in Fig. 2 by using either a normal exon 1 probe (pUCLYXh16), as indicated on the lefthand side, or a probe derived from the Raji translocated *myc* allele [the *Sac* II (Sc) fragment indicated at the bottom of the righthand panel]. After hybridization of the filters with their respective probes, washing was carried out at 65°C in 15 mM sodium chloride/1.5 mM sodium citrate, pH 7/0.1% $NaDodSO_4$. T represents the position of the *Bgl* II (B) fragment containing the Raji translocated c-*myc* and N represents the position of the *Bgl* II fragment containing the normal c-*myc* allele. Xh = *Xho* I.

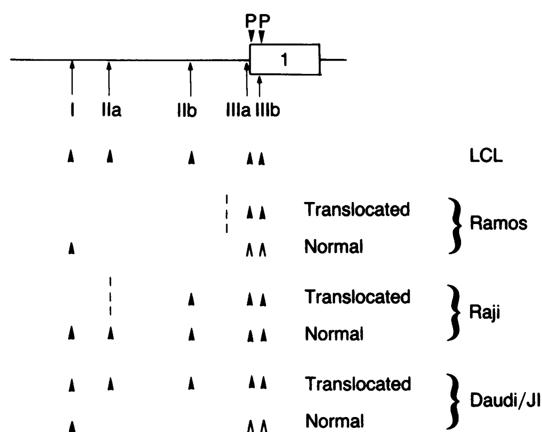

FIG. 5. Distribution of major hypersensitive sites in c-*myc* chromatin in BLs and LCLs. P = promoter site. The vertical dashed lines near Ramos and Raji translocated c-*myc* genes represent the positions of the breakpoints. Closed arrowheads indicate sites established in these experiments; open arrowheads indicate uncertainty about sites.

with the two promoters of the *myc* gene (sites IIIa and IIIb) are not altered in sensitivity when there is preferential use of the two promoters (e.g., DHLCL and HMy2). The previously studied case of hypersensitive sites in a BL cell line (10) in which translocation occurs upstream of all five chromatin sites and brings the immunoglobulin μ enhancer near the breakpoint shows a qualitatively similar pattern to that seen here. Taken together it seems likely that the presence of the μ enhancer near to the translocation point has little effect on the hypersensitivity map, and cases in which breakpoints are near (Raji or Ramos) or far (Daudi or JI) from the *myc* gene do not have much difference in their patterns. If any of these chromatin sites is altered by translocation it must be at a subtle level below the detection of this assay.

Interestingly, the Raji translocated c-*myc* gene breaks close to or within the hypersensitive site IIa. This may be related to the mechanism of this translocation but any relationship is speculative. More interesting is the detection of new hypersensitive sites within the immunoglobulin gene region adjacent to the translocation point in Raji. This indicates that new regions of gene control are present near c-*myc* after translocation and may play a role in the aberrant expression of this gene. It is noteworthy that a consistent feature of translocations in BLs seems to be that the immunoglobulin constant region gene is rearranged near the breakpoint [whether it be 5′ (21, 24) or 3′ (22, 26, 27) of c-*myc*]. This may argue that the constant region gene has associated sequences involved in maintenance rather than elevation of immunoglobulin gene transcription. After translocation, such putative controls may be brought to bear on the c-*myc* gene, thereby causing unregulated, sustained expression rather than elevated expression of this gene.

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1. Alitalo, K., Schwab, M., Lin, C. C., Varmus, H. E. & Bishop, J. M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1707–1711.
2. Little, C. D., Nau, M. M., Carney, D. N., Gazdar, A. F. & Minna, J. D. (1983) *Nature (London)* **306**, 194–196.
3. Klein, G. (1981) *Nature (London)* **294**, 313–318.
4. Dalla-Favera, R., Bregni, M., Erikson, J., Patterson, D., Gallo, R. C. & Croce, C. M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7824–7827.
5. Taub, R., Kirsch, I., Morton, C., Lenoir, G., Swan, D., Tronick, S., Aaronson, S. & Leder, P. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7837–7841.
6. Neel, B. G., Jhanwar, S. C., Chaganti, R. S. K. & Hayward, W. S. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7842–7846.
7. Hamlyn, P. H. & Rabbitts, T. H. (1983) *Nature (London)* **304**, 135–139.
8. Taub, R., Moulding, C., Battey, J., Murphy, W., Vasicek, T., Lenoir, G. M. & Leder, P. (1984) *Cell* **36**, 339–348.
9. Tuan, D. & London, I. M. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 2718–2722.
10. Siebenlist, V., Hennighausen, L., Battey, J. & Leder, P. (1984) *Cell* **37**, 381–391.
11. Wu, C. (1980) *Nature (London)* **286**, 854–860.
12. Stalder, J., Groudine, M., Dodgson, J. B., Engel, J. D. & Weintraub, H. (1980) *Cell* **19**, 973–980.
13. Varmus, H. E., Quintrell, N. & Wyke, J. (1981) *Virology* **108**, 28–46.
14. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
15. Denhardt, D. T. (1966) *Biochem. Biophys. Res. Commun.* **23**, 641–646.
16. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
17. Rabbitts, T. H., Forster, A., Hamlyn, P. & Baer, R. (1984) *Nature (London)* **309**, 592–597.
18. Vieira, J. & Messing, J. (1982) *Gene* **19**, 259–268.
19. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
20. Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. J. H. & Roe, B. A. (1980) *J. Mol. Biol.* **143**, 161–178.
21. Bernard, O., Cory, S., Gerondakis, S., Webb, E. & Adams, J. M. (1983) *EMBO J.* **2**, 2375–2383.
22. Davis, M., Malcolm, S. & Rabbitts, T. H. (1984) *Nature (London)* **308**, 286–288.
23. Wiman, K. G., Clarkson, B., Hayday, A. C., Saito, H., Tonegawa, S. & Hayward, W. S. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6798–6802.
24. Rabbitts, T. H., Hamlyn, P. H. & Baer, R. (1983) *Nature (London)* **306**, 760–765.
25. Persson, H. & Leder, P. (1984) *Science* **225**, 718–720.
26. Croce, C. M., Thierfelder, W., Erikson, J., Nishikura, K., Finan, J., Lenoir, G. M. & Nowell, P. C. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6922–6926.
27. Hollis, G. F., Mitchell, K. F., Battey, J., Potter, H., Taub, R., Lenoir, G. M. & Leder, P. (1984) *Nature (London)* **307**, 752–755.