Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 3170–3174, May 1984
Genetics

Translocated c-*myc* oncogene of Burkitt lymphoma is transcribed in plasma cells and repressed in lymphoblastoid cells

(gene regulation/c-*myc* deregulation/enhancers/genetics of B-cell neoplasia/B-cell differentiation)

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Communicated by Hilary Koprowski, February 8, 1984

ABSTRACT **We examined somatic cell hybrids between Burkitt lymphoma cells and either human lymphoblastoid cells or mouse plasmacytoma cells for the expression of the translocated c-*myc* oncogene. The results of this study indicate that the translocated c-*myc* oncogene is transcribed in plasma cells but is repressed in lymphoblastoid cells. Thus, the factors necessary for translocated c-*myc* transcription are present in plasma cells and Burkitt lymphoma cells but are absent or inactive in lymphoblastoid cells. Since the distance between the rearranged immunoglobulin loci and the c-*myc* oncogene can even exceed 30–50 kilobases, we speculate that the translocated c-*myc* oncogene is under the transcriptional control of enhancer-like elements capable of acting over long distances. The activity of this long-range enhancer may depend on the interaction with transacting factors that are active in plasma cells and in Burkitt lymphoma cells but are not active in lymphoblastoid cells. We also examined the transcription of the first exon of the c-*myc* oncogene, which becomes separated from the second and third exon because of the chromosomal break involving the first intron. This exon is transcribed at high levels in ST486 Burkitt lymphoma cells with the t(8;14) chromosome translocation. Hybrids between lymphoblastoid and ST486 cells expressed high levels of transcripts of the first exon, whereas hybrids between plasma cells and ST486 cells did not. Thus, transcription of the separated first exon can be enhanced in lymphoblastoid and Burkitt lymphoma cells because of its close proximity to the heavy chain enhancer that is normally located between the joining and the switch region of the C_μ gene. Such enhancement, however, does not occur in plasma cells, possibly because these cells are able to suppress completely the c-*myc* oncogene, unless it has been placed in the proximity of a rearranged immunoglobulin constant region gene.**

We have shown that the c-*myc* oncogene translocates from its normal position on band q24 of chromosome 8 to the heavy chain locus on chromosome 14 in Burkitt lymphomas with the t(8;14) chromosome translocation, while the c-*myc* oncogene remains on chromosome 8 and either the λ or the κ chain locus translocates to a region distal to the c-*myc* oncogene in Burkitt lymphoma with the t(8;22) and t(2;8) translocations, respectively (1–5). Independently of whether it is structurally rearranged, the c-*myc* oncogene involved in the translocation is transcriptionally active, while the c-*myc* oncogene on the normal chromosome 8 is transcriptionally silent (3–7). We have also shown that while the c-*myc* oncogene involved in the translocation escapes normal transcriptional control, the untranslocated c-*myc* oncogene on normal chromosome 8 is repressed in plasma cells (3–7). To determine whether the activation of the translocated c-*myc* oncogene depends on the differentiated state of the B cells harboring the c-*myc* oncogene involved in the translocation, we have hybridized different human Burkitt lymphoma cells with human lymphoblastoid cells and with mouse plasmacytoma cells and examined the hybrids for the expression of the normal and of the involved c-*myc* oncogene.

In addition, since we have found that the first exon of the c-*myc* oncogene, which is separated from the c-*myc* coding exons by the chromosomal break occurring in the first c-*myc* intron, is located 5′ of the heavy chain enhancer and is transcriptionally very active in ST486 Burkitt lymphoma cells (7), we have also examined the hybrids between ST486 and either lymphoblastoid or plasmacytoma cells for the expression of the separated first exon to determine whether its transcriptional activity depends on the differentiated state of the hybrids.

MATERIALS AND METHODS

Cells. Human GM1500-6TG-OUB lymphoblastoid cells are derived from the Epstein–Barr virus-transformed cell line GM1500-6TG (8, 9) and are deficient in hypoxanthine phosphoribosyltransferase and resistant to 0.1 mM ouabain. The GM1500-6TG cell line is a 6-thioguanine-resistant mutant of the Epstein–Barr virus-transformed GM1500 cell line (8, 9). We have tested these three lymphoblastoid cell lines for tumorigenicity by injecting 10^7 cells per mouse into BALB/c nude mice. No tumors were obtained in three of three mice injected with GM1500 cells, which were observed for 91 days. Only one of five mice injected with GM1500-6TG cells developed a tumor (0.8 cm in diameter), which appeared 64 days after injection. The five mice were observed for 103 days. All three mice injected with GM1500-6TG-OUB cells developed tumors within 23 days after injection, which were larger than 1.5 cm in diameter 70 days after injection. All three lymphoblastoid cell lines carry a germline c-*myc* oncogene.

Three Burkitt lymphoma cell lines with the t(8;14) chromosome translocations were studied: Daudi, CA46, and ST486. Daudi cells contain a translocated but unrearranged c-*myc* oncogene on the $14q^+$ chromosome (1–3). CA46 and ST486 cells carry a translocated and altered c-*myc* oncogene, which is rearranged head-to-head with one of the immunoglobulin heavy chain constant region genes on the $14q^+$ chromosome (2, 7). In both cell lines the rearrangement occurred within the first c-*myc* intron (2, 7). We have previously shown that ST486 cells, in addition to transcribing the translocated coding exons of the c-*myc* oncogene, also express high levels of transcripts of the first exon that has been separated from the coding exons by the chromosomal breakpoint. Each of the three Burkitt lymphoma cell lines was fused with the GM1500-6TG-OUB lymphoblastoid cell line in the presence of polyethylene glycol 1000 according to standard procedures (10) and the hybrids were selected in HAT medium (hypoxanthine/aminopterin/thymidine) (11) containing 0.01 mM ouabain (12). ST486 cells were also hybridized with NP3 mouse plasmacytoma cells (3–6) and the

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Abbreviations: bp, base pair(s); kb, kilobase(s).

hybrids were selected in HAT medium containing 0.01 mM ouabain (12).

Expression of Immunoglobulin Chains. Parent and hybrid cells were grown for 5–8 hr in leucine-deficient medium or methionine-deficient medium containing 5% dialyzed fetal calf serum and [^{3}H]leucine (70 Ci/mmol) at 100 μCi/ml or [^{35}S]methionine (40 Ci/mmol) at 100 μCi/ml (1 Ci = 37 GBq). The expression of human immunoglobulin chains was determined by immunoprecipitation of either culture fluids or cytoplasmic extracts of the hybrid and parental cells by using rabbit antibodies specific for human immunoglobulin chains, followed by the addition of 50 μl of a 10% suspension of fixed *Staphylococcus aureus* as described (1, 3–5). Labeled immunoglobulin chains were then separated by Na-DodSO$_4$/polyacrylamide gel electrophoresis (13, 14).

DNA Gel Electrophoresis and Southern Transfer. Agarose gel (0.7% or 1%) electrophoresis was carried out in 40 mM Tris·HCl/5 mM NaOAc/2.0 mM EDTA, pH 8.0. *Hin*dIII-digested λ phage DNA molecular weight markers (0.75 μg per lane) (Bethesda Research Laboratories) were included on every gel. Cellular DNA samples were digested with restriction enzymes and then subjected to electrophoresis in a horizontal agarose (Sigma) slab gel (10 μg of DNA per lane). Transfer of DNA from gel to nitrocellulose sheet (Millipore) was performed essentially as described by Southern (15).

RNA Transfer. Cytoplasmic RNA was extracted by the cesium chloride method as described (16). RNA was denatured in 1 M glyoxal in 10 mM NaPO$_4$ (pH 6.5) at 50°C for 1 hr, electrophoresed in a 1% agarose gel, transferred to nitrocellulose, prehybridized, and hybridized to 0.2 μg of the *myc* probe (about 4×10^7 cpm) according to the method of Thomas (17). Twenty micrograms of RNA was loaded in each lane. Prehybridizations and hybridizations were performed as reported (3, 7). Molecular weight markers were electrophoresed in each gel.

Probes. The first exon probe was an *Xho* I–*Pvu* II leader fragment of 449 base pairs (bp) within exon I (7). The probe for the coding exons of the c-*myc* gene was a 1029-bp fragment obtained from the *Pst* I digestion of a cDNA clone (pRyc 7.4), which includes 221 bp of the 3′ end of the exon II and all of exon III (6, 7). The probe used for S1 mapping analysis to detect novel initiation sites or cryptic splicing sites within the intervening sequences between the first and second exons was a double-stranded 1.4-kilobase (kb) *Xba* I–*Bst*EII fragment, 5′ ^{32}P-labeled at the *Bst*EII site within the second exon (7). The immunoglobulin C_μ gene probe was a 1.2-kb *Eco*RI genomic probe described elsewhere (3).

S1 Nuclease Analysis of Human c-*myc* Transcripts. Nuclease S1 analysis was carried out according to Sharp *et al.* (18) with modifications (19) by using 5′ end-labeled human c-*myc* DNA clones (6). The 5′ ^{32}P-end-labeled DNA probes were heat-denatured, hybridized in 80% deionized formamide to 20 μg of cytoplasmic RNA at 55°C for 10 hr, digested with 80 units of S1 nuclease (P-L Biochemicals), and analyzed by electrophoresis on a 7 M urea/4% polyacrylamide gel (20). The DNA probe was 5′ end-labeled by the method of Maxam and Gilbert (21).

RESULTS

Expression of Immunoglobulin Genes in Hybrids Between Human Lymphoblastoid and Burkitt Lymphoma Cells. The hybrids between GM1500-6TG-OUB cells and the three different Burkitt lymphoma cells, Daudi, CA46 and ST486, were morphologically identical to the lymphoblastoid parental cells and grew in large clumps like lymphoblastoid cells (data not shown). As shown in Fig. 1*A*, the hybrids between Daudi cells and GM1500-6TG-OUB cells expressed immunoglobulin chains of both parental cells. Daudi cells expressed two κ chains (P33 and κ') (Fig. 1*A*, lane 1) (22). The P33

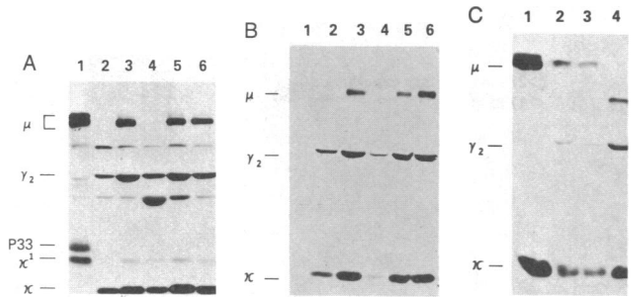

FIG. 1. Expression of immunoglobulin chains in Burkitt lymphoma × human lymphoblastoid cell hybrids. (*A*) Immunoprecipitation of the cytosol of parental and hybrid cells using an anti-human immunoglobulin antiserum. Lane 1, Daudi cells; lane 2, GM1500 cells; lanes 3–6, Daudi × GM1500-6TG-OUB, hybrids 223-A5, 223-C4, 223-B3, and 223-B4, respectively. (*B*) Immunoprecipitation of the culture fluids of the same parental and hybrid cells as in *A*. (*C*) Immunoprecipitation of the cytosol of ST486 cells, GM1500-6TG-OUB cells, and their hybrids using an anti-human immunoglobulin antiserum. Lane 1, ST486 cells; lanes 2 and 3, hybrids 286-2D3 and 286-1C1, respectively; lane 4, GM1500-6TG-OUB.

chain is a highly glycosylated form of the aberrant κ' chain (22). All four hybrids expressed the κ chains and the γ_2 chains of the GM1500-6TG-OUB parent and the κ' chains of the Daudi parent. Hybrids 223-A5, 223-B3, and 223-B4 also expressed the μ chain of the Daudi parent (Fig. 1*A*, lanes 3, 5, and 6, respectively), while hybrid 223-C4 lost the ability to produce μ chain (Fig. 1*A*, lane 4) due to chromosomal segregation (data not shown). Daudi cells expressed only membrane-bound IgM and did not secrete IgM (Fig. 1*B*, lane 1) (22). By contrast the hybrids produced only the secreted form of IgM (Fig. 1 *A* and *B*).

All four hybrids (267-B1, 267-BC4, 267-AC4, and 267-AD5), between CA46 and GM1500-6TG cells, also expressed the immunoglobulin chains of both parental cells (data not shown).

As shown in Fig. 1*C*, the two hybrids between ST486 and GM1500-6TG-OUB expressed the γ_2 chains of the GM1500-6TG-OUB parent and μ chains of the ST486 parent (Fig. 1*C*, lanes 2 and 3).

Presence of the c-*myc* Oncogene in the Hybrids Between Human Lymphoblastoid and Burkitt Lymphoma Cells. Since Daudi cells contain a translocated c-*myc* gene in its germ-line configuration that cannot be distinguished from the normal c-*myc* gene on the normal chromosomes 8 of Daudi cells and of GM1500-6TG-OUB cells, we examined the Daudi × GM1500-6TG-OUB hybrids (223-A5, 223-C4, 223-B3, and 223-B4) for the presence of the rearranged μ chain gene that is located on the 14q$^+$ chromosome of Daudi cells (14). This gene, which is contained within the 18-kb *Bam*HI restriction fragment band, was present in all four of the hybrid clones examined (data not shown), indicating that the 14q$^+$ chromosome was present in all four hybrid clones. As shown in Fig. 2*A*, two hybrids between CA46 and GM1500-6TG-OUB cells (267-B1 and AC4) lost the translocated and rearranged c-*myc* oncogene (lanes 2 and 4), while hybrid 267-BC4 (lane 3) and hybrid 267-AD5 (data not shown) retained the translocated and rearranged c-*myc* oncogene in addition to the germ-line gene.

As shown in Fig. 2*B*, both hybrids (286-1C1 and 286-2D3) between ST486 and GM1500-6TG cells retained the germ-line and the rearranged and translocated c-*myc* oncogene (lanes 2 and 3, respectively).

Expression of *myc* Transcripts in Hybrid Cells. The GM1500-6TG-OUB lymphoblastoid cells expressed high levels of transcripts of the c-*myc* oncogene (Fig. 3, lanes 3 and 4). The 6-thioguanine-resistant mutant GM1500-6TG line

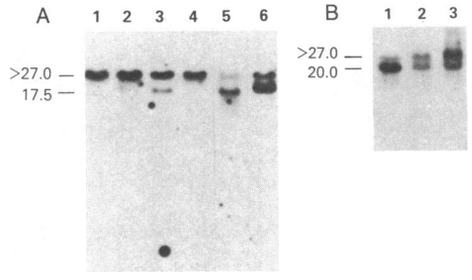

FIG. 2. Southern blotting analysis of *Bam*HI-digested parental and hybrid cell DNAs using the *myc* (Ryc 7.4) cDNA probe. (*A*) Lane 1, GM1500-6TG-OUB DNA; lanes 2–4, CA46 × GM1500-6TG-OUB hybrid 267-AC4, 267-BC4, and 267-B1 DNAs, respectively; lane 5, CA46 DNA; lane 6, ST486 DNA. (*B*) Lane 1, ST486 DNA; lane 2, hybrid 286-2D5 DNA; lane 3, hybrid 286-1C1 DNA. Sizes are shown in kb.

from which the ouabain-resistant mutant GM1500-6TG-OUB was derived expressed lower levels of *myc* transcripts (Fig. 3, lane 2). On the contrary, the parental GM1500 human lymphoblastoid cell line expressed very low levels of *myc* transcripts (Fig. 3, lane 1). Interestingly, the levels of c-*myc* transcripts in the three lymphoblastoid cell lines correlated with their tumorigenicity in nude mice (see *Materials and Methods*).

As shown in Fig. 4*A*, all of the hybrids between Daudi cells and GM1500-6TG-OUB cells expressed levels of *myc* transcripts lower than in the Daudi parental cells. The hybrids between CA46 and GM1500-6TG cells also expressed levels of *myc* transcripts much lower than in the CA46 parent (Fig. 4*B*). In these hybrids the levels of *myc* transcripts were even lower than in the GM1500-6TG-OUB parent. The hybrids between ST486 and GM1500-6TG-OUB cells also expressed levels of c-*myc* transcripts much lower than both parental cells (Fig. 4*C*, left).

Since it is possible to distinguish between the transcripts of the normal and of the translocated and rearranged c-*myc* gene by using the first intron probe (7), we examined the hybrids between CA46 and GM1500-6TG-OUB and the hybrids between ST486 and GM1500-6TG-OUB for the expression of either the germ-line or the rearranged and translocated c-*myc* gene. As shown in Fig. 5, all of the lymphoblastoid Burkitt hybrids that retained both the germ-line and the rearranged c-*myc* oncogene expressed only the normal 322-nucleotide band and not the translocated 930-, 810-, and 770-nucleotide band c-*myc* oncogene transcripts (Table 1).

Thus, we conclude that while the normal c-*myc* oncogene is expressed, the translocated c-*myc* oncogene is repressed in lymphoblastoid cells (Fig. 5 and Table 1), and the juxtaposition of the translocated c-*myc* oncogene to the heavy chain locus is insufficient to activate c-*myc* transcription.

To be activated, the translocated c-*myc* oncogene must be

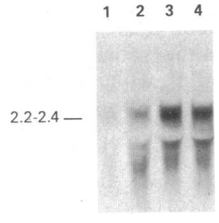

FIG. 3. RNA transfer blotting analysis of the RNA of the GM1500 cell lines using the c-*myc* cDNA probe (Ryc 7.4). Lane 1, GM1500 cell RNA; lane 2, GM1500-6TG cell RNA; lane 3, GM1500-6TG-OUB RNA; lane 4, RNA derived from a tumor induced in nude mice by injection of GM1500-6TG-OUB cells. Sizes are shown in kb.

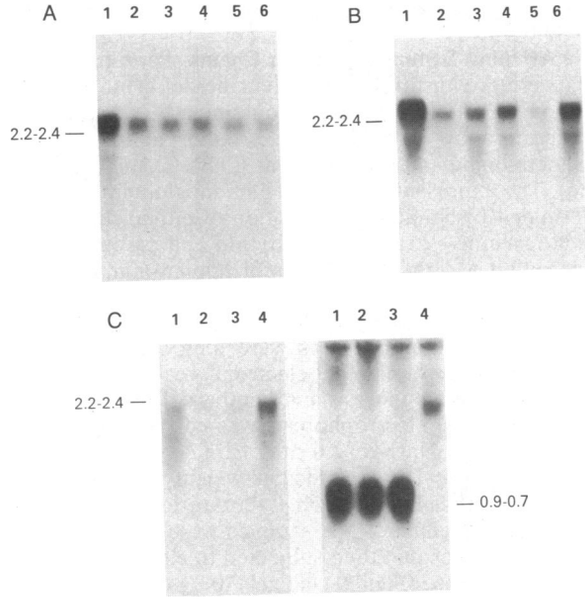

FIG. 4. RNA transfer blotting analysis of RNA of hybrids between Burkitt lymphoma and GM1500-6TG-OUB lymphoblastoid cells. (*A*) The blot was hybridized with the c-*myc* cDNA (Ryc 7.4) specific for the second and third exon. Lane 1, Daudi RNA; lanes 2–5, hybrid 223-A5, 223-C4, 223-B3, and 223-B4 DNAs, respectively; lane 6, GM1500 RNA. (*B*) The blot was hybridized with the Ryc 7.4 probe. Lane 1, CA46 RNA; lanes 2–5, hybrid 267-BC4, 267-AC4, 267-B1, and 267-AD5 RNAs, respectively; lane 6, GM1500-6TG-OUB RNA. (*C*) Left, the blot was hybridized with the Ryc 7.4 probe; lane 1, ST486; lanes 2 and 3, hybrid 286-2D3 and 286-1C1 RNAs, respectively; lane 4, GM1500-6TG-OUB RNA. Right, the blot was hybridized with the c-*myc* first exon probe; lane 1, ST486 RNA; lanes 2 and 3, hybrid 286-2D3 and 286-1C1 RNAs, respectively; lane 4, GM1500-6TG-OUB RNA. Sizes are shown in kb.

present in Burkitt cells or in plasma cells, since we have previously shown that the translocated c-*myc* oncogene is expressed in a plasmacytoma background (4–6). To confirm this observation, we hybridized ST486 Burkitt lymphoma cells with NP3 mouse myeloma cells and examined hybrids for the expression of the translocated and of the normal c-*myc* oncogene. As shown in Fig. 6 *A* and *B*, hybrids containing the translocated and the normal c-*myc* oncogene expressed only the translocated c-*myc* oncogene and not the normal c-*myc* gene transcripts. Thus, the normal and the translocated c-*myc* oncogene are regulated differently in lymphoblastoid cells and in plasma cells (Table 1).

Because we observed previously that the c-*myc* first exon, which is separated by the chromosome break, is transcribed at high levels in ST486 Burkitt lymphoma cells (7), we also examined the two ST486 × GM1500-6TG-OUB hybrids for the expression of the two short transcripts derived from the separated first *myc* exon (0.9–0.7 kb). As shown in Fig. 4*C* (right), the hybrids expressed very high levels of transcripts of the first exon.

We have also examined the transcription of the separated first c-*myc* exon in hybrids between NP3 and ST486 cells. As shown in Fig. 6*B*, the plasma cell hybrids containing the separated first exon of the human c-*myc* gene failed to transcribe it (Table 1).

DISCUSSION

The results of the analysis of somatic cell hybrids between Burkitt lymphoma and human lymphoblastoid cells indicate that these hybrids, which are morphologically identical to the lymphoblastoid parental cells, transcribe the normal c-*myc* oncogene but not the translocated c-*myc* oncogene. On

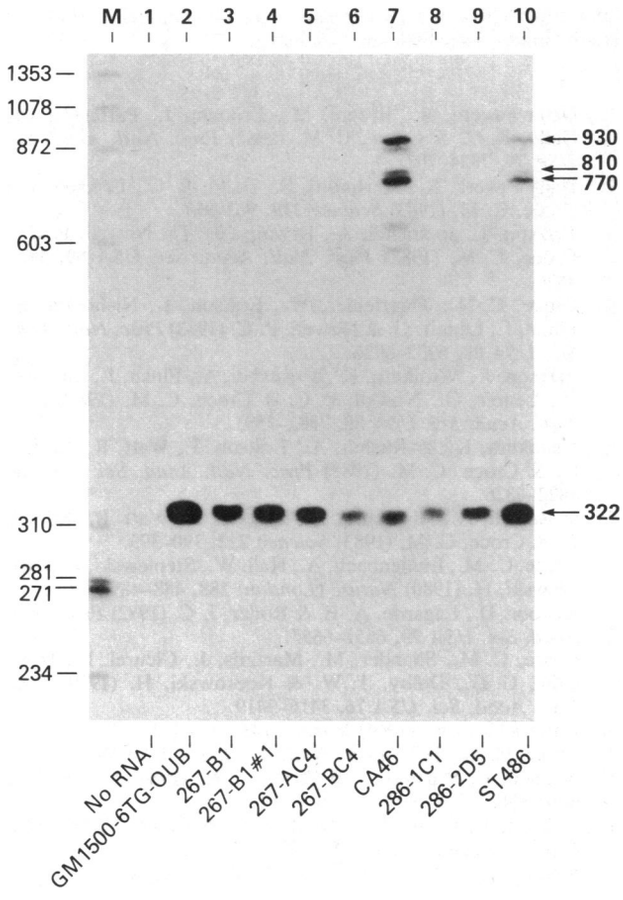

FIG. 5. S1 nuclease mapping analysis for aberrant c-*myc* transcripts in the hybrid cells between GM1500-OUB and Burkitt lymphoma cell lines. S1 probe was prepared from the human genomic c-*myc* clone pc-*myc* 41·HE (7). A DNA fragment, *Xba* I–*Bst*EII, encompassing a part of the first intron and the second exon, 5′ ^{32}P-labeled at the 5′ end of the *Bst*EII site, was used as an S1 probe. Conditions for S1 nuclease analysis were as described (6). Hybrid 267-B1#1 was derived from a tumor obtained from injecting hybrid 267-B1 into a nude mouse. The results with hybrid 267-AD5 were essentially identical to those obtained with hybrid 267-BC4 (data not shown). Sizes of bands are shown in numbers of nucleotides.

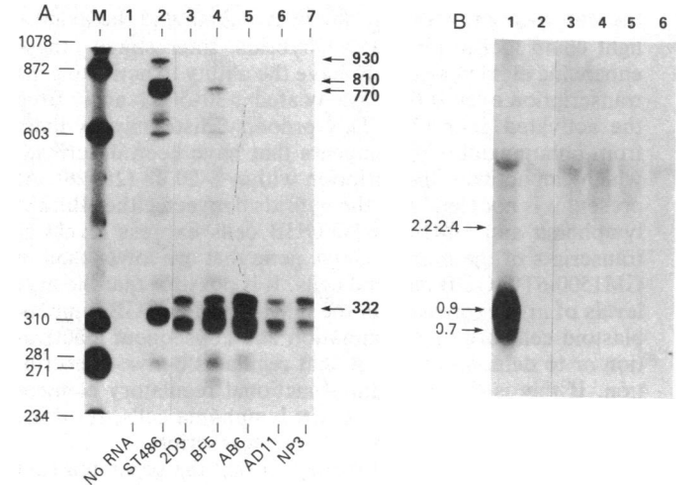

FIG. 6. S1 nuclease mapping analysis for aberrant c-*myc* transcripts in the hybrids between NP3 and Burkitt lymphoma cell lines. The double-stranded *Xba* I–*Bst*EII DNA fragment prepared from clone pc-*myc* 41·HE, ^{32}P-labeled at the 5′ end of the *Bst*EII site, was used as an S1 probe. Conditions for S1 nuclease analysis were as described (6). Four hybrid subclones of the NP3 × ST486 hybrid 287-2D3 were studied by S1 nuclease analysis (lanes 3–6). Subclones 2D3 and AD11 contained the untranslocated germ-line c-*myc* but have lost the translocated c-*myc* coding exons. They did not express human c-*myc* transcripts (lanes 3 and 6). Subclone AB6 has lost both human c-*myc* genes (lane 5). Subclone BF5 contained both the translocated and the germ-line c-*myc* oncogene and expressed the translocated and rearranged c-*myc* gene. Sizes are shown in nucleotides. (*B*) RNA transfer blotting analysis of hybrids between ST486 and NP3 using a probe specific for the first exon of the c-*myc* oncogene. Lane 1, ST486 RNA; lanes 2–5, hybrids between ST486 and NP3 cells that contain the separated first exon of the c-*myc* oncogene; lane 6, NP3 RNA. Hybrid subclone 287-2D3 BF5 RNA is in lane 3. Since we do not observe the 2.2- to 2.4-kb *myc* RNA, we can conclude that the hybrid does not express the normal *myc* gene. Sizes are shown in kb.

the contrary, hybrids between Burkitt lymphoma and plasma cells, which are morphologically identical to the plasmacytoma parental cells, express the translocated c-*myc* oncogene but not the untranslocated gene on the normal chromosome 8. Thus, we postulate the existence of cell type-specific transacting factors, which are active in plasma cells and Burkitt lymphoma cells but are inactive or absent in lymphoblastoid cells and which interact with the rearranged immunoglobulin loci and the c-*myc* oncogene located in their proximity, resulting in an open chromatin configuration and consequently in the activation of the c-*myc* oncogene involved in the translocation.

Thus, in addition to the immunoglobulin heavy chain gene enhancer (23), which is separated from the c-*myc* oncogene in some Burkitt lymphomas with a head-to-head rearrangement between the c-*myc* oncogene and one of the heavy chain genes (7), a different class of transcriptional enhancing elements must exist. Such enhancing elements must be present within all three immunoglobulin loci since we observe enhanced transcription of the c-*myc* oncogene involved in

Table 1. Transcripts of the human c-*myc* gene in B-cell hybrids

Cells	Human c-*myc* gene*			Transcripts of the		
	Germ-line	Rearranged and translocated	Separated first exon*	Human germ-line c-*myc* gene	Rearranged and translocated human c-*myc* gene	Separated first exon of the human c-*myc* gene
ST486	+	+	+	−	+	+
GM1500-6TG-OUB	+	−	−	+	−	−
286-2D5	+	+	+	+	−	+
286-1C1	+	+	+	+	−	+
NP3	−	−	−	−	−	−
287-2D3-BF5	+	+	+	−	+	−

*Cellular DNAs were cut with *Hin*dIII and, after agarose gel electrophoresis and Southern blotting, the nitrocellulose filters were hybridized with a probe specific for either the second and third exon or the first exon of the c-*myc* oncogene. The separated first exon appears as a 4.7-kb band after hybridization with the first exon probe.

the translocation with both the heavy chain and the κ and λ light chain loci in plasma cell hybrids. Interestingly, these enhancing elements seem to have the ability to activate gene transcription even if they are located >30–50 kb away from the activated gene (24). This property distinguishes them from immunoglobulin enhancers that have been described, which can activate transcription within 5–20 kb (25, 26). At present it is not clear why the hybrids between either Burkitt lymphoma and GM1500-6TG-OUB cells express levels of transcripts of the normal c-*myc* gene that are lower than in GM1500-6TG-OUB parental cells. It is possible that the high levels of *myc* expression in the GM1500-6TG-OUB lymphoblastoid cells are due to mutation and consequent inactivation or to deletion of a gene that regulates c-*myc* transcription. If this is the case, this functional regulatory element could be provided by the Burkitt lymphoma cells, resulting in a decrease of the levels of c-*myc* transcripts.

Of considerable interest is the fact that the separated first exon of the c-*myc* oncogene of ST486 cells is transcribed at high levels in ST486 cells and in hybrids with lymphoblastoid cells but is shut off in hybrids with plasma cells. Since the enhancer between the joining and the switch region of the μ gene is located 3′ of this exon in ST486 cells (2, 7), it is likely that this enhancer is responsible for the high levels of transcripts of the first exon. However, transcription of the ST486 *myc* first exon does not occur in plasma cells. Thus, it seems likely that plasma cells produce factors capable of inactivating the *myc* oncogene on normal chromosome 8. It is not clear, however, why in ST486 Burkitt lymphoma cells the normal c-*myc* gene is repressed and the separated first exon is expressed (7). It is possible that the close proximity of the heavy chain enhancer to the separated first exon can overcome the transcription block to which the normal c-*myc* oncogene is subjected, in Burkitt lymphoma cells but not in plasma cells.

Recently, two reports by Leder *et al.* (27) and by Saito *et al.* (28) have proposed two different models to explain the involvement of the c-*myc* oncogene in Burkitt lymphomas. Leder *et al.* have suggested that rearrangements involving the first exon of the c-*myc* oncogene or its separation from the two coding exons result in its failure to respond to a repressor capable of shutting off the expression of the gene. This model is inconsistent with our findings that the rearranged c-*myc* gene of Burkitt lymphoma is repressed in lymphoblastoid cells. If the deregulation of the c-*myc* oncogene were due to the structural alteration of the gene, the c-*myc* gene should have been expressed in the lymphoblastoid cells. In addition, this model and the translational control model proposed by Saito *et al.* (27) fail to consider that in many Burkitt lymphomas with the t(8;14) translocation the involved c-*myc* gene is not rearranged (1–5) and that in the variant lymphomas with the t(2;8) and t(8;22) translocations, the rearrangement does not involve sequences 5′ of the coding exons but occurs distally (3′) to the c-*myc* oncogene (4, 5).

We thank Gerald Vuocolo and Steven Goldflam for expert technical assistance and Ms. Kathleen Pinette for preparation of this manuscript. This research was supported by National Institutes of Health Grants GM 31060 and CA 16685.

1. Dalla Favera, R., Bregni, M., Erikson, J., Patterson, D., Gallo, R. C. & Croce, C. M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7824–7827.
2. Dalla Favera, R., Martinotti, S., Gallo, R. C., Erikson, J. & Croce, C. M. (1983) *Science* **219,** 963–967.
3. Erikson, J., ar-Rushdi, A., Drwinga, H. L., Nowell, P. C. & Croce, C. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4822–4826.
4. Croce, C. M., Thierfelder, W., Erikson, J., Nishikura, K., Finan, J., Lenoir, G. & Nowell, P. C. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 6922–6926.
5. Erikson, J., Nishikura, K., ar-Rushdi, A., Finan, J., Emanuel, B., Lenoir, G., Nowell, P. C. & Croce, C. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7581–7585.
6. Nishikura, K., ar-Rushdi, A., Erikson, J., Watt, R., Rovera, G. & Croce, C. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4822–4826.
7. ar-Rushdi, A., Nishikura, K., Erikson, J., Watt, R., Rovera, G. & Croce, C. M. (1983) *Science* **222,** 390–393.
8. Croce, C. M., Linnenbach, A., Hall, W., Steplewski, Z. & Koprowski, H. (1980) *Nature (London)* **288,** 488–489.
9. Kozbor, D., Lagarde, A. E. & Roder, J. C. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6651–6655.
10. Croce, C. M., Shander, M., Martinis, J., Cicurel, L., D'Ancona, G. G., Dolby, T. W. & Koprowski, H. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3416–3419.
11. Littlefield, J. W. (1964) *Science* **145,** 709–710.
12. Croce, C. M. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 315–319.
13. Erikson, J., Martinis, J. & Croce, C. M. (1981) *Nature (London)* **294,** 173–175.
14. Erikson, J., Finan, J., Nowell, P. C. & Croce, C. M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 5611–5615.
15. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
16. ar-Rushdi, A., Tan, K. B. & Croce, C. M. (1982) *Somatic Cell Genet.* **8,** 151–161.
17. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
18. Sharp, R. A., Berk, A. J. & Berget, S. M. (1980) *Methods Enzymol.* **65,** 750–768,
19. Weaver, R. F. & Weissman, C. (1979) *Nucleic Acids Res.* **7,** 1175–1193.
20. Maniatis, T., Jeffrey, A. & Van Sande, H. (1975) *Biochemistry* **14,** 3783–3794.
21. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
22. Erikson, J. & Croce, C. M. (1982) *Eur. J. Immunol.* **12,** 697–701.
23. Mercola, M., Wang, X., Olsen, J. & Calame, J. (1983) *Science* **221,** 663–665.
24. Emanuel, B. S., Selden, J. R., Chaganti, R. S. K., Jhanwar, S., Nowell, P. C. & Croce, C. M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2444–2446.
25. Khoury, G. & Gruss, P. (1983) *Cell* **33,** 313–314.
26. Picard, D. & Schaffner, W. (1984) *Nature (London)* **307,** 80–82.
27. Leder, P., Battey, J., Lenoir, G., Maulding, G., Murphy, W., Potter, H., Stewart, T. & Taub, R. (1983) *Science* **222,** 765–771.
28. Saito, H., Hayday, A. C., Wimar, K., Hayward, W. S. & Tonegawa, S. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7476–7480.