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Translocation of an immunoglobulin κ locus to a region 3′ of an unrearranged c-*myc* oncogene enhances c-*myc* transcription

[Burkitt lymphoma/t(2;8) chromosome translocation/oncogene activation/genetics of B-cell neoplasia]

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ABSTRACT **We have studied somatic cell hybrids between mouse myeloma and JI Burkitt lymphoma cells carrying a t(2;8) chromosome translocation for the expression of human κ chains and for the presence and rearrangements of the human c-*myc* oncogene and κ chain genes. Our results indicate that the c-*myc* oncogene is unrearranged and remains on the 8q$^+$ chromosome of JI cells. Two rearranged C_κ genes were detected: the expressed allele on normal chromosome 2 and the excluded κ allele that was translocated from chromosome 2 to the involved chromosome 8 (8q$^+$). The distribution of V_κ and C_κ genes in hybrid clones retaining different human chromosomes indicated that C_κ is distal to V_κ on 2p and that the breakpoint in this Burkitt lymphoma is within the region carrying V_κ genes. High levels of transcripts of the c-*myc* gene were found when it resided on the 8q$^+$ chromosome but not on the normal chromosome 8, demonstrating that translocation of a κ locus to a region distal to the c-*myc* oncogene enhances c-*myc* transcription.**

In Burkitt lymphoma cells carrying the t(8;14) chromosome translocation, the c-*myc* oncogene translocates from its normal location on chromosome 8 (1–3) to the heavy chain locus (1) on chromosome 14 (4). In some of these Burkitt lymphomas, the translocated c-*myc* oncogene is not rearranged within a large *Bam*HI restriction fragment, whereas in others, it is rearranged head-to-head (5′ to 5′) with the C_μ gene (1, 3, 5, 6). In either case, translocation of the c-*myc* oncogene enables it to escape from normal transcriptional control and the gene is expressed constitutively at high levels (7, 8), while the c-*myc* oncogene on normal chromosome 8 is transcriptionally silent (8). No difference in the c-*myc* product has been detected in comparing normal lymphocytes to Burkitt lymphoma cells with or without a rearranged c-*myc* gene (9).

Recently we examined Burkitt lymphoma cells carrying the variant t(8;22) chromosome translocation by the somatic cell genetics approach and found that the c-*myc* oncogene remains on the involved chromosome 8 while the C_λ locus translocates to a region 3′ of the c-*myc* oncogene (10). In this case, the unrearranged c-*myc* oncogene, which is proximal (5′) to the translocated C_λ locus, becomes transcriptionally highly active, while the c-*myc* oncogene on the normal chromosome 8 becomes inactive (10). These findings indicate that placement of rearranged immunoglobulin genes either proximal (5′) or distal (3′) to a c-*myc* oncogene allows constitutive expression of the oncogene at high levels (8, 10).

In this study, we have investigated Burkitt lymphoma cells carrying the t(2;8) translocation (11) to determine the orientation of the κ chain genes on the short arm of chromosome 2 (12) and to establish the mode of c-*myc* activation in these cells.

MATERIALS AND METHODS

Cells. JI Burkitt lymphoma cells (11) were fused with NP3 nonproducer mouse myeloma cells (13) in the presence of polyethylene glycol 1,000 as described (14). Independent hybrid clones were picked up and expanded for chromosomal, DNA, and RNA analysis. One of the clones, JI 4-5, was also subcloned by limiting dilution in nonselective medium and subclones were picked up and expanded. Three subclones, JI-45B7, JI-45B9, and JI-45H11, were also analyzed.

Isozyme Analysis. Hybrids were studied for the expression of human malate dehydrogenase 1 and isocitrate dehydrogenase 1, which are markers for the short and long arms of human chromosome 2, respectively, by starch gel electrophoresis (4).

Chromosome Analysis. Parental and hybrid cell chromosomes were studied by the trypsin/Giemsa banding method as described (7, 13). At least 25 metaphases were examined for each hybrid and scored only if there was adequate banding of human chromosomes. The G11 technique was used to confirm the human origin of relevant chromosomes (7, 13).

Analysis of Immunoglobulin Chains. Parent and hybrid cells were grown in methionine-deficient medium containing 5% dialyzed fetal calf serum and 100 μCi of [^{35}S]methionine (400 Ci/mmol; 1 Ci = 3.7×10^{10} Bq) per ml for 5–8 hr. The expression of human κ chain was determined by immunoprecipitation of culture fluids or of cytoplasmic extracts of the hybrid and parental cells by using rabbit anti-human κ chain-specific antibodies followed by the addition of 50 μl of a 10% suspension of fixed *Staphylococcus aureus* as described (14). Labeled immunoglobulin chains were then separated by NaDodSO$_4$/polyacrylamide gel electrophoresis (14).

Southern Blotting Analysis. Agarose gel (0.7% or 1%) electrophoresis was carried out in 40 mM Tris/5 mM NaOAc/2.0 mM EDTA, pH 8.0. *Hin*dIII-digested λ phage DNA (0.75 μg per lane) (Bethesda Research Laboratories) molecular weight markers were included on every gel. Cellular DNA samples were digested with *Bam*HI, *Eco*RI, or *Hin*dIII and subjected to electrophoresis in a horizontal agarose (Sigma) slab gel (10 μg of DNA per lane). Gels were stained for 10 min with ethidium bromide (1 μg/ml) and photographed under UV light. Transfer of DNA from gel to nitrocellulose sheet (Millipore) was performed essentially as described by Southern (15).

Preparation of Labeled Probe DNAs. The c-*myc* probe (Ryc 7.4) was a cDNA clone (8, 14) derived from a K562 cDNA li-

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Abbreviation: kb, kilobase(s).

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brary (16). The c-*mos* probe (PAB) was a genomic clone of the human c-*mos* gene (17). The C_κ probe was a cDNA clone in M13 mp7. The clone is a *Mbo* III–*Hin*dIII fragment equivalent to codon 115 until the poly(A) tail of mRNA. The V_κ probe (HK 101/80) was a genomic clone in pBR322 (18). The DNA probes were labeled with ^{32}P by the nick-translation procedure (19) and had specific activities of 0.3–2 × 10^8 cpm/0.2 μg of DNA. DNA polymerase I was purchased from Boehringer Mannheim; [^{32}P]-NTPs were from Amersham.

Hybridization. DNA on nitrocellulose sheets was hybridized to ^{32}P-labeled probe DNA in a hybridization solution containing 50% (vol/vol) formamide as described (13, 14). After hybridization, the filters were washed, air-dried, and exposed to XRP-5 film for various periods.

S1 Nuclease Analysis of Human c-*myc* Transcripts in Hybrid Cells. S1 nuclease analysis was carried out according to Sharp and Berk *et al.* (20) with modifications (8) using a 5′-end-labeled human c-*myc* cDNA clone (Ryc 7.4) (8). Cytoplasmic RNA was prepared by the cesium chloride method (21). The DNA probe was 5′-end-labeled by the method of Maxam and Gilbert (22). 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 (8). The relative amount of c-*myc* RNA in various cells was estimated by quantitative S1 nuclease mapping followed by scanning of autoradiograms exposed for various times.

RESULTS

Chromosomal Analysis. As shown in Fig. 1*A*, JI Burkitt lymphoma cells carry the t(2;8) variant chromosome translocation. Other changes, including trisomy 15 and 21, a marker derived from centric fusion of two 21s, an unidentified small acrocentric marker, and a derivative chromosome 6 resulting in partial trisomy for 1q, were also present in varying proportions of the cells. We analyzed independent NP3 mouse myeloma × JI Burkitt lymphoma hybrid cells for the presence of the normal chromosomes 2 and 8 and for the $2p^-$ and $8q^+$ chromosomes that result from the reciprocal translocation between chromosomes 2 and 8. Table 1 shows the results of the cytogenetic analysis of primary hybrid clones and of two hybrid JI 4-5 subclones, with those showing segregation of the $2p^-$ and $8q^+$ chromosomes being of particular interest (Fig. 1 *B* and *C*).

Immunoglobulin κ Genes in JI × NP3 Hybrids. To determine the orientation and organization of the V_κ and the C_κ genes on human chromosome 2 and in the 2;8 translocation, hybrids

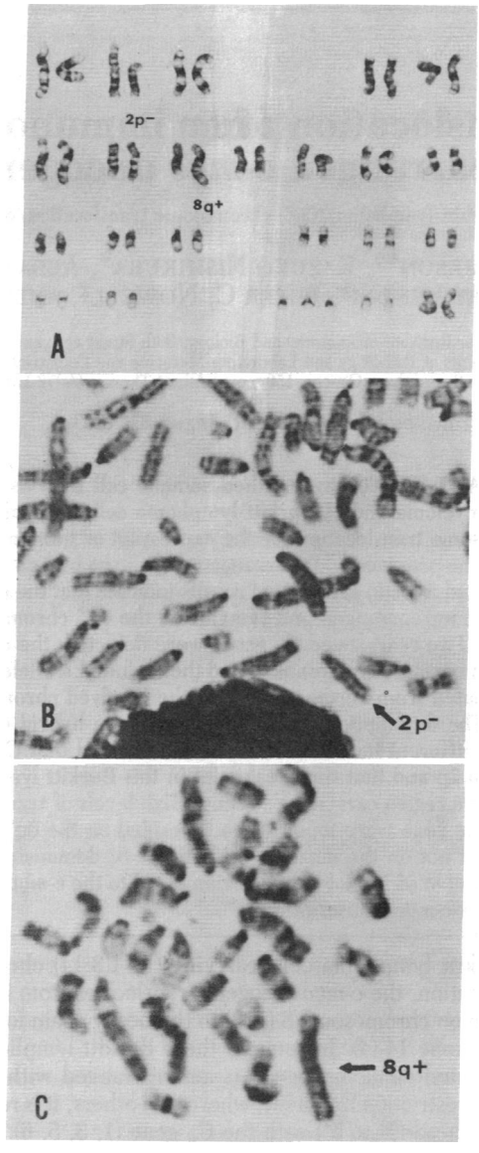

FIG. 1. (*A*) Representative karyotype of parental JI cell line with 2;8 translocation and additional changes, including trisomy 21 and a $6q^+$ chromosome resulting in partial trisomy for 1q. (*B*) Portion of a trypsin/Giemsa-banded metaphase from hybrid 5-4 that retained human chromosomes $2p^-$ (arrow) and 8 but had no normal chromosome 2 or $8q^+$. (*C*) Portion of a trypsin/Giemsa-banded metaphase from hybrid 4-5 that had the $8q^+$ human chromosome (arrow) but had no chromosome 2, $2p^-$, or normal 8.

Table 1. Human κ genes and oncogenes in JI × NP3 hybrids

Cells	Human chromosome*				Human isozyme		Human κ chain expression	Human gene C_κ			Human oncogene		Human c-*myc* transcript
	8	$8q^+$	2	$2p^-$	MDH	IDH		15 kb	7.5 kb	V_κ	c-*mos*	c-*myc*	
JI	++	++	++	++	+	+	+	+	+	+	+	+	+++
JI 4-5	−	++	−	−	+	−	−	+	−	+	+	+	+++
JI 4-5B7	−	+	−	−	+	−	−	+	−	+	+	+	++
JI 4-5H11	−	−	−	−	−	−	−	−	−	−	−	−	−
JI 5-4	+	−	−	++	−	+	−	−	−	+	+	+	−
JI 6-5	−	−	++	−	+	+	+	−	+	+	−	−	−
JI 4-2L	−	++	−	−	+	−	−	+	−	+	+	+	+++
NP3	−	−	−	−	−	−	−	−	−	−	−	−	−

MDH, malate dehydrogenase; IDH, isocitrate dehydrogenase. kb, kilobases.
*Frequency of metaphases with relevant chromosomes: −, none; +, 10–30%; ++, >30%.

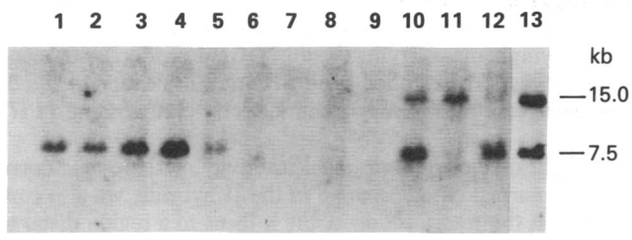

FIG. 2. Southern blotting analysis of NP3 × JI somatic cell hybrids (lanes 2–12) for the presence of human C_κ genes after *Bam*HI digestion. Lane 1, DNA from the NP3 mouse myeloma parent; lane 13, JI Burkitt lymphoma DNA; lane 2, hybrid JI 6-5 DNA; lane 7, hybrid JI 5-4 DNA; and lane 11, hybrid JI 4-5 DNA.

and parental cells were also studied for the presence and rearrangements of immunoglobulin κ chain genes. Southern blotting analysis after digestion of JI cell DNA with *Bam*HI, which cuts outside the C_κ gene, revealed a 15-kb and a 7.5-kb C_κ band in these cells. The germ line C_κ gene is 12 kb in size after *Bam*HI digestion (data not shown). The hybrid (JI 6-5) that retained the *Bam*HI 7.5-kb C_κ band was found to produce human κ chains (Fig. 2, Table 1), whereas the hybrids retaining only the 15-kb κ band (JI 4-2L, JI 4-5, and JI 4-5B7) did not express human κ chains (Fig. 3, Table 1). Thus, we conclude that the 7.5-kb band represents the expressed κ allele and the 15-kb band represents the excluded κ allele.

Chromosomal analysis of these hybrids (Table 1) indicated that expression of κ chains and the presence of the *Bam*HI 7.5-kb C_κ band were associated with the presence of the normal chromosome 2, whereas the excluded C_κ allele was associated with the $8q^+$ chromosome. These data indicate that the C_κ locus is translocated to chromosome 8 in JI Burkitt lymphoma cells carrying the t(2;8) translocation. Because hybrids with either the $2p^-$ chromosome or the $8q^+$ chromosome retained human V_κ genes in the absence of normal chromosome 2 (Fig. 4, Table 1), we also conclude that in JI Burkitt lymphoma cells the chromosome breakage occurred between V_κ genes (Table 1), which are located proximal to the C_κ locus on chromosome 2p.

Localization of the c-*myc* Oncogene in JI Cells. As shown in Fig. 5, the c-*myc* oncogene is unrearranged within a large (≈28-kb) *Bam*HI fragment in JI Burkitt lymphoma cells. Analysis of the NP3 × JI hybrids indicated that the c-*myc* gene segregated with the $8q^+$ chromosome and not with the $2p^-$ chromosome (Table 1). Furthermore, there was no segregation in the hybrid clones between c-*myc* and c-*mos*, an oncogene located on band q22 of chromosome 8 (2) (Table 1). Taken together, these findings indicate that in JI cells the c-*myc* oncogene remains on chromosome 8 and the C_κ gene moves from

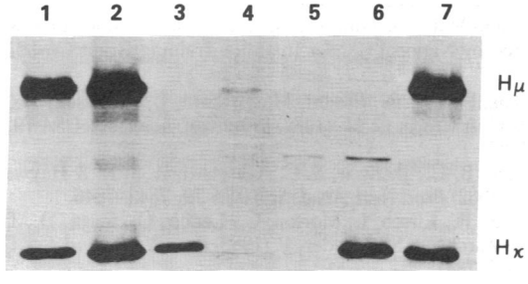

FIG. 3. $NaDodSO_4$/polyacrylamide gel electrophoresis of human immunoglobulins immunoprecipitated from culture supernatants from a non-Burkitt type undifferentiated lymphoma, JD38 (lane 1), from a human lymphoblastoid line, GM607 (lane 2), and from JI × NP3 hybrids (lanes 3–6). Lane 3, JI 6-5; lane 4, JI 5-4; lane 5, JI 4-5; and lane 7, JI parental cells.

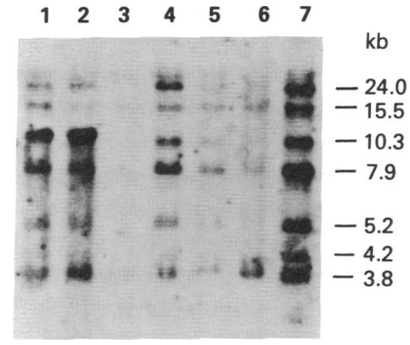

FIG. 4. Southern blotting analysis of NP3 × JI somatic cell hybrids for the presence of human V_κ genes after *Bam*HI digestion. Lane 3, DNA from the NP3 mouse myeloma parent; lane 7, JI DNA; lane 1, hybrid JI 6-5 DNA; lane 2, hybrid JI 5-4 DNA; lane 4, hybrid JI 4-5 DNA; and lane 6, hybrid JI 4-2L DNA.

chromosome 2 to a region of chromosome 8 distal (3′) to this oncogene. Because the c-*myc* gene is oriented with its 5′ exon proximal and its 3′ exon distal to the centromere on chromosome 8 (10), we conclude that the 5′ end of the C_κ gene is in close proximity to the 3′ end of the c-*myc* oncogene on the $8q^+$ chromosome.

Transcription of the c-*myc* Oncogene in NP3 × JI Hybrids. S1 nuclease protection procedures have proven useful in the detection and quantitation of levels of human c-*myc* transcripts in mouse × human hybrids (8, 10). As shown in Fig. 6 and Table 1, only JI hybrids that contained the $8q^+$ chromosome expressed high levels of human c-*myc* transcripts, whereas hybrids containing the normal chromosomes 8 and the $2p^-$ without the $8q^+$ did not. This result indicates that transcription of the human c-*myc* gene that remains on the $8q^+$ chromosome is enhanced by the translocation of the κ locus to a region 3′ of the oncogene. Interestingly, the data show that the c-*myc* gene on the normal chromosome 8 is transcriptionally silent. No expression of the human c-*mos* gene was detected in hybrids containing either the normal chromosome 8 or the $8q^+$ (data not shown).

DISCUSSION

The results presented in this paper indicate that the breakpoint in JI Burkitt lymphoma cells carrying the t(2;8) translocation is within the immunoglobulin κ locus and that the C_κ gene and some of the V_κ genes translocate to chromosome 8 (Fig. 7). Further, the V_κ genes are proximal and the C_κ gene is distal on band p11 of chromosome 2. Because the c-*myc* oncogene remains on the chromosome 8 involved in the 2;8 translocation and because elevated levels of human c-*myc* transcripts were

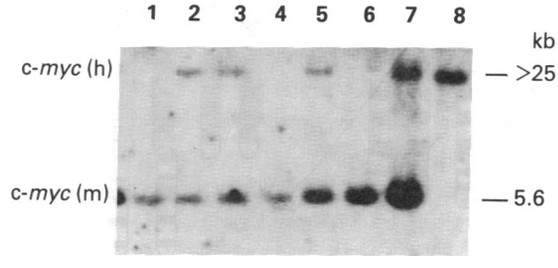

FIG. 5. Southern blot hybridization of *Bam*HI-digested NP3 × JI hybrid DNAs with the c-*myc* probe (Ryc 7.4). Lane 1, NP3 mouse parental DNA; lane 8, human JI DNA; lane 2, hybrid JI 4-5 B9 DNA that has retained the 8 and the $8q^+$ chromosomes; lane 3, hybrid JI 5-4 DNA; lane 5, hybrid JI 4-5 DNA; lane 6, hybrid JI 6-5 DNA; and lane 7, hybrid JI 4-2L DNA.

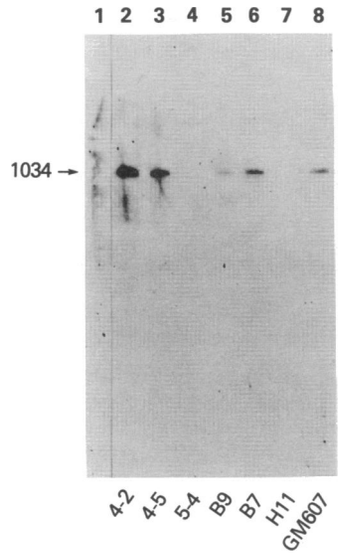

FIG. 6. S1 nuclease protection experiment using the Ryc 7.4 DNA probe (8). The human c-*myc* RNA protects a DNA segment 1,034 nucleotides long (8). Lane 1, no RNA control; lane 2, JI 4-2L RNA; lane 3, JI 4-5 RNA; lane 4, JI 5-4 RNA; lane 5, RNA from hybrid JI 4-5B9 subclone that has retained the $8q^+$ in <10% of the cells; lane 6, JI 4-5B7 RNA; lane 7, RNA from hybrid JI 4-5 H11 subclone that has lost the $8q^+$; and lane 8, RNA from GM607 human lymphoblastoid cells (8). The levels of expression of *myc*-RNA in JI cells were similar to the levels detected in hybrid JI 4-2L (data not shown).

detectable only when the $8q^+$ chromosome was present in the hybrid clones derived from JI cells, we conclude that enhancement of c-*myc* transcription observed in this Burkitt lymphoma carrying the t(2;8) translocation (8) results from the translocation of the κ locus to chromosome 8. This finding contrasts with Burkitt lymphomas carrying the t(8;14) translocation, where enhanced c-*myc* transcription results from translocation of the c-*myc* oncogene to the heavy chain locus on chromosome 14 (7, 8).

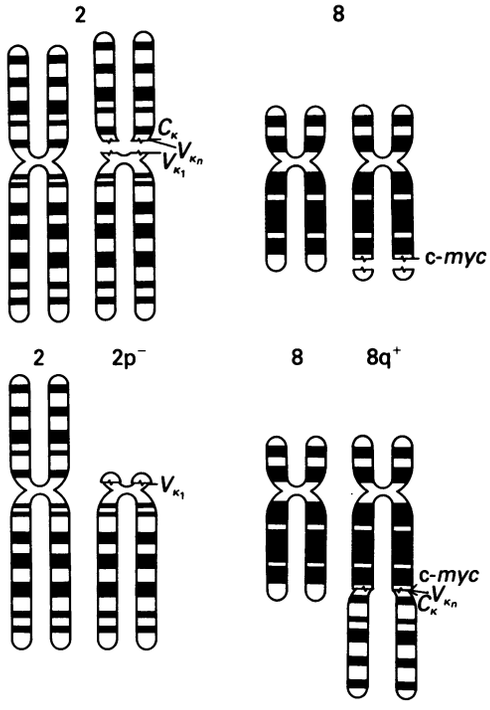

FIG. 7. Diagram of the t(2;8) translocation occurring in 8% of Burkitt lymphomas. As shown, the V_κ genes are proximal and the C_κ gene is distal on band p11 of chromosome 2. While most of the V_κ genes stay on the $2p^-$ chromosome, the C_κ gene translocates to the involved chromosome 8 ($8q^+$). The c-*myc* oncogene remains on the involved chromosome 8 ($8q^+$).

In light of the transcriptional inactivity of the c-*myc* oncogene on normal chromosome 8 in the JI hybrids, we also conclude that the translocation of the κ locus to a region distal to the c-*myc* locus removes this oncogene from normal transcriptional control. We have described similar findings in Burkitt lymphomas with the t(8;14) translocation (8) and with the t(8;22) chromosome translocation, where the C_λ locus translocates to a region distal to the c-*myc* locus on chromosome 8 (10). Thus, either translocation of the c-*myc* oncogene to the heavy chain locus or translocation of the light chain loci to a region distal to the c-*myc* oncogene results in the activation of the c-*myc* oncogene flanking the translocation breakpoint, while the c-*myc* oncogene on the normal chromosome 8 is repressed.

It appears that the juxtaposition of the c-*myc* oncogene and human immunoglobulin genes, independent of a head-to-head or head-to-tail rearrangement, is sufficient to enhance transcription of the c-*myc* oncogene adjacent to the breakpoint. The same sequences that normally enhance immunoglobulin gene transcription may be responsible for the enhancement of c-*myc* transcription in the Burkitt lymphoma. The involvement of immunoglobulin enhancers localized between the J_H and the switch region of heavy chains (23, 24) in c-*myc* activation has been postulated (24); however, these do not seem likely candidates in Burkitt lymphoma cells with the t(8;14) translocation and c-*myc* rearrangements, because these enhancers translocate to the $8q^-$ chromosome and are not 5′ to the translocated and activated c-*myc* oncogene in these cells (25). The existence of the immunoglobulin enhancer sequences in the κ light chain gene has also been suggested (26, 27). In this case, the enhancer may be located in the J_κ–C_κ intron (about 700 base pairs 5′ to the C_κ region) (25) or in the 3′ one-third of the κ gene (>2.6 kb downstream of J_κ) (26). It is possible that κ chain enhancer sequences may be involved in activation of the c-*myc* gene in JI Burkitt lymphoma because these sequences translocate to the c-*myc* oncogene on chromosome 8. In all cases examined thus far, it appears that the critical association in Burkitt lymphoma cells is between the oncogene and the 5′ side of the constant region genes, even though they may remain separated to some degree by intervening variable regions and other sequences.

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