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Transcriptional activation of an unrearranged and untranslocated c-*myc* oncogene by translocation of a C_λ locus in Burkitt lymphoma cells

[immunoglobulin genes/t(8;22) chromosome translocation/oncogene activation/B-cell neoplasia]

CARLO M. CROCE*, WILLIAM THIERFELDER*, JAN ERIKSON*, KAZUKO NISHIKURA*, JANET FINAN†, GILBERT M. LENOIR‡, AND PETER C. NOWELL†

*The Wistar Institute of Anatomy and Biology, 36th and Spruce Streets, Philadelphia, PA 19104; †Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104; and ‡International Agency for Research on Cancer, 150, cours Albert Thomas, 69372 Lyon cedex 2, France

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ABSTRACT **We have studied somatic cell hybrids between mouse myeloma cells and IARC-BL2 Burkitt lymphoma human cells carrying a t(8;22) chromosome translocation for the presence and expression of human immunoglobin λ chains and for the c-*myc* oncogene. The results indicate that the c-*myc* oncogene remains on the 8q+ chromosome and that the excluded and rearranged C_λ allele translocates from chromosome 22 to this chromosome 8. As a result of the translocation, transcriptional activation of the c-*myc* oncogene on the rearranged chromosome 8 (8q+) occurs, while the c-*myc* oncogene in the normal chromosome 8 is transcriptionally silent. These findings suggest that the translocation of a rearranged immunoglobulin locus to the 3′ side of an unrearranged c-*myc* oncogene may enhance its transcription and contribute to malignant transformation.**

Translocation of the same segment of human chromosome 8, 8q24→qter, to chromosome 14, 2, or 22 occurs in Burkitt lymphoma (1–5). Chromosomes 14, 2, and 22 are known to carry the genes for immunoglobulin heavy, κ, and λ chains, respectively (6–11). Recent studies have indicated that the c-*myc* oncogene, which normally resides on band q24 of chromosome 8 (12, 13), translocates to the immunoglobulin heavy chain gene cluster in those Burkitt lymphomas with the usual t(8;14) chromosome translocation (12–15). In some cases a head-to-head rearrangement between the C_μ and the c-*myc* genes is observed, in others the c-*myc* oncogene is in its germ-line configuration (12–15). Regardless of whether it is rearranged, the translocated c-*myc* oncogene is transcriptionally very active (16, 17), whereas the untranslocated c-*myc* oncogene on the normal chromosome 8 is transcriptionally silent in Burkitt lymphoma cells and in derived hybrids between mouse myeloma and Burkitt cells (17, 18). In addition, we have also shown that in Burkitt lymphoma cells in which the breakpoint occurred between the c-*myc* first and second exon, both segments of the decapitated c-*myc* oncogene are transcriptionally active (18). This finding indicates that enhancing elements, which activate the transcription of the c-*myc* gene, are located on both sides of the breakpoint in the heavy chain locus on chromosome 14 in some Burkitt lymphoma cell lines and suggests that enhancement of c-*myc* transcription may occur even when immunoglobulin gene sequences are placed 3′ of the c-*myc* promoter (18).

In the present study, we have investigated the organization of the c-*myc* and the C_λ genes in Burkitt lymphoma cells carrying the t(8;22) chromosome translocation in order to determine the mechanisms of c-*myc* activation in Burkitt lymphoma cells carrying one of the infrequent variant chromosome translocations.

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MATERIALS AND METHODS

Cells. IARC-BL2 Burkitt lymphoma cells (BL2) carry a t(8;22) chromosome translocation, do not harbor the Epstein–Barr virus, and express immunoglobulin λ chains (19). These cells were fused with NP3 mouse myeloma cells (20) in the presence of polyethylene glycol 1000 as described (20). Hybrids were selected in hypoxanthine/aminopterin/thymidine (HAT) medium (21) containing 10 μM ouabain (22).

Chromosome Analysis. Parental and hybrid cell chromosomes were studied by the trypsin/Giemsa banding method as described (16, 20). At least 20 metaphase spreads were examined for each hybrid, and they were scored only if there was adequate banding of human chromosomes. Selected metaphases were studied by the G-11 technique to confirm the human origin of relevant chromosomes (16, 20).

Expression of Immunoglobulin Chains. Parental and hybrid cells were grown in leucine-deficient medium containing 5% dialyzed fetal calf serum and [^{3}H]leucine (70 Ci/mmol; 1 Ci = 3.7×10^{10} Bq) at 100 μCi/ml for 4 hr. The expression of human Ig chains was determined by immunoprecipitation of culture fluids or cytoplasmic extracts of the hybrid and parental cells, using rabbit antibodies specific for human immunoglobulins followed by the addition of 50 μl of a 10% suspension of fixed *Staphylococcus aureus* as described (6, 9, 23). Labeled immunoglobulin chains were then separated by NaDodSO$_4$/polyacrylamide gel electrophoresis (16).

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 phage λ 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, and *Hin*dIII restriction endonucleases and then 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 (24).

Preparation of Labeled Probe DNAs. The c-*myc* probe (Ryc 7.4) was a cDNA clone (16) derived from a K562 cDNA library (25). The c-*mos* probe (PAB) was a genomic clone of the human c-*mos* gene (26). The C_λ probe was a genomic clone (Chr 22 λ 5) of the C_λ gene in λ*WES*. This clone contains an 8.0-kilobase

Abbreviation: kb, kilobase(s).

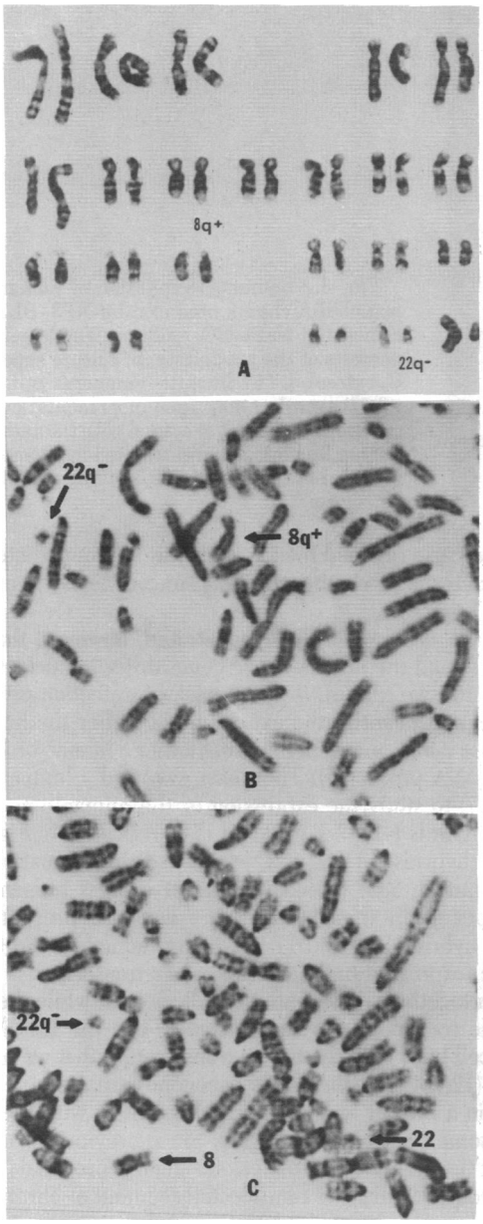

FIG. 1. (*A*) Representative karyotype of parental BL2 cell line with 8;22 translocation and additional changes resulting in partial trisomy for 1q and 7q: 46,XY,t(8;22)(q24;q11), −1, +der(1), t(1;7)(q32;q11), −6, +der(6), t(1;6)(q21;q25). (*B*) Portion of a trypsin/Giemsa-banded metaphase from hybrid 17-6 that retained human chromosomes 8q+, 22q− (arrows), and 22 but had no normal chromosome 8. (*C*) Portion of a trypsin/Giemsa-banded metaphases from hybrid 3-1 that had human chromosomes 8, 22, and 22q− (arrows) but no 8q+.

(kb) *Eco*RI fragment that includes Ke^- Oz^- and Ke^- Oz^+ (10). The DNA probes were labeled with ^{32}P by the nick-translation procedure (27) and had specific activities of 0.3–2 × 10^8 cpm/0.1 μg of DNA. DNA polymerase I was purchased from Boehringer Mannheim; [α-^{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 (16, 20). After hybridization, the filters were washed, air dried, and exposed to Kodak XRP-5 film for various periods.

S1 Nuclease Analysis of Human and Mouse c-*myc* Transcripts in Hybrid Cells. Cytoplasmic RNA was extracted by the cesium chloride method (28).

S1 nuclease analysis was carried out according to Sharp *et al.* (29) with modifications (17), using a 5′-end-labeled human c-*myc* cDNA (Ryc 7.4) clone (16, 17). The probe was 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 (17). The DNA probe was 5′-end-labeled by the method of Maxam and Gilbert (30). The relative amount of c-*myc* RNA in various cells was estimated by quantitative S1 nuclease mapping followed by the scanning of suitably exposed autoradiograms.

RESULTS

Chromosomal Localization of C_λ DNA Sequences. As shown in Fig. 1, BL2 Burkitt lymphoma cells carry a t(8;22) chromosome translocation. As a result of the reciprocal translocation, two marker chromosomes are generated: an 8q+ chromosome with the translocated q11→qter of chromosome 22, and a 22q− chromosome with the translocated region q24→qter of chromosome 8. A combination of cytogenetic analysis and Southern blotting studies of DNA from the parental cells and derived hybrids was used to determine the chromosomal localization, arrangement, and expression of the C_λ genes in these tumor cells.

Southern blotting data from BL2 cell DNA, digested with *Eco*RI, indicated that this Burkitt lymphoma cell line carries two rearranged λ chain genes, represented by the 12.0-kb (middle) and 6.8-kb (lower) bands in Fig. 2. Studies of the NP3–BL2 hybrids, summarized in Table 1, showed that the 6.8-kb (lower) band was present only in those hybrids that expressed λ chains (Fig. 3) and that also contained the normal chromosome 22 (Table 1).

Conversely, the 12.0-kb (middle) band was present in the two hybrids (3-1, 3-2) that neither expressed λ chains nor had the normal chromosome 22. Thus, the 12.0-kb (middle) band appears to correspond to the unexpressed (excluded) allele, and the 6.8-kb (lower) band represents the expressed allele, located on the normal chromosome 22. These findings parallel our pre-

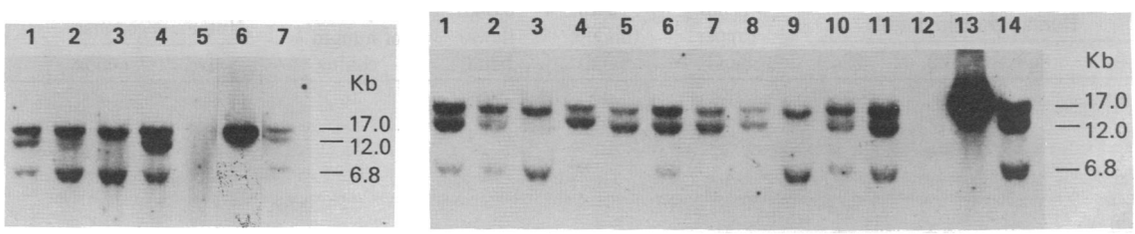

FIG. 2. Southern blotting analysis of NP3–BL2 somatic cell hybrids after *Eco*RI digestion of cellular DNA. DNA on the nitrocellulose filters was hybridized with a C_λ genomic clone. (*Left*) Lanes 1–4, NP3–BL2 hybrid DNAs. Hybrid 17-6 DNA is in lane 1; hybrid 2-35 DNA is in lane 2; hybrid 3-2 DNA is in lane 3. Lane 5, NP3 mouse myeloma DNA; lane 6, PAF simian virus 40-transformed human cell DNA; lane 7, BL2 Burkitt lymphoma DNA. (*Right*) Lanes 1–11, NP3–BL2 hybrid DNAs. Hybrid 1-15 DNA is in lane 5, hybrid 1-18 DNA is in lane 7, and hybrid 1-23 DNA is in lane 8. Hybrid 3-1 DNA is in lane 3 and hybrid 1-9 DNA is in lane 9. NP3 mouse myeloma DNA is in lane 12, PAF DNA is in lane 13, and BL2 parental DNA is in lane 14.

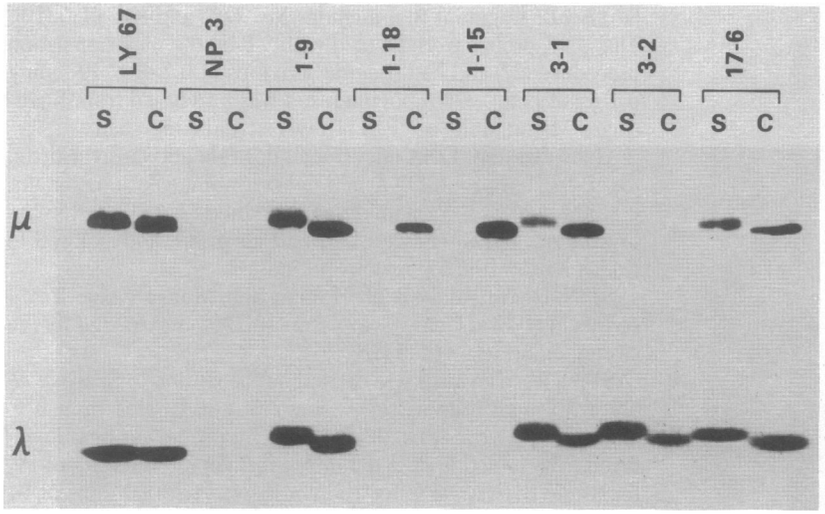

FIG. 3. Immunoprecipitation of human immunoglobulin chains produced by NP3–BL2 hybrid clones and $NaDodSO_4$/polyacrylamide gel electrophoresis of the precipitate. S, culture supernatant; C, cytosol. LY67 Burkitt lymphoma cells carry a t(8;22) translocation. Immunoprecipitation of BL2 culture supernatant or cytosol with the antiserum to human immunoglobulin resulted in a band pattern identical to that of hybrids 1-9, 3-1, and 17-6 (data not shown).

vious observations on Burkitt lymphomas with the t(8;14) translocation, which showed that the expressed immunoglobulin allele is on the chromosome not involved in the translocation (16, 20).

The present data also indicated that the unproductively rearranged C_λ gene is on the segment of chromosome 22 that is translocated to chromosome 8 in BL2 cells. As shown in Table 1, three clones (1-9, 3-1, and 3-2) had lost the 8q+ chromosome but retained the 22q−; and all three clones had also lost the 12.0-kb (middle) band corresponding to the excluded allele, indicating its location on the 8q+ chromosome rather than on the 22q−. These results are in agreement with *in situ* hybridization data of de la Chapelle *et al.* (31).

It is also apparent from Fig. 2 and Table 1 that both the translocated segment of chromosome 22 and the normal chromosome 22 in BL2 cells also contain unrearranged C_λ genes (17-kb, upper band), reflecting the fact that several copies of λ genes are normally present on chromosome 22 (10).

Chromosomal Localization and Expression of the c-*myc* Oncogene. We have previously shown that the c-*myc* oncogene, which is located on band q24 of chromosome 8, translocates to the deleted chromosome 14 in those Burkitt lymphomas with the t(8;14) chromosome translocation (12, 16), and it shows increased expression in its new location (17, 18). In the case of hybrids between BL2 and NP3, we found that clones 4-35 and 17-6, which retained the 8q+ chromosome but not the normal 8, contained not only the c-*mos* oncogene, which is located on band q22 of chromosome 8 (32), but also the c-*myc* oncogene in its germ-line configuration (Fig. 4 and Table 1). Because these two clones also retained the 22q−, it was not immediately possible to determine whether the c-*myc* oncogene had been translocated.

We have also previously demonstrated, however, that since the mouse and the human c-*myc* genes differ in nucleotide sequence (15), we can use the S1 nuclease protection procedure to detect and quantify the expression of either the human or the mouse c-*myc* oncogene by using either a human or a mouse c-*myc* cDNA probe (17). Therefore, we used a human cDNA probe (17) to study the expression of the human c-*myc* oncogene in hybrids 1-15, 3-1, 3-2, and 17-6. As shown in Fig. 5 and Table 1, hybrids 3-1 and 3-2, which had the normal chromosome 8 but no 8q+ (Table 1), did not express human c-*myc* transcripts, even though the 22q− was also present. Conversely, hybrids 1-15 and 17-6, which retained the 8q+ chromosome, expressed high levels of c-*myc* transcripts.

Taken together, these findings indicate that while the c-*myc* oncogene translocates to chromosome 14 in the Burkitt lymphoma cell lines with the t(8;14) translocation that we have examined (12, 14–16), the c-*myc* oncogene remains on chromosome 8 in a Burkitt lymphoma cell line, BL2, with the t(8;22) chromosome translocation (Fig. 6). Since previous results suggest that the 5′ end of the c-*myc* oncogene is proximal (18, 20), we can conclude that the translocated segment of chromosome 22 must be placed distal (3′) to the intact c-*myc* oncogene on the rearranged chromosome 8 (Fig. 6). Furthermore, it appears that the translocation of the C_λ locus to a DNA region located 3′ of the c-*myc* oncogene results in the enhancement of c-*myc* transcription in these neoplastic B cells.

Table 1. Immunoglobulin genes and oncogenes in BL2 hybrids

Cell line	Human chromosomes*				Human C_λ genes			Expression of human λ chains	Human oncogenes		Transcripts of human c-*myc*
	8	8q+	22	22q−	17-kb (upper band)	12-kb (middle band)	6.8-kb (lower band)		c-*myc*	c-*mos*	
BL2	+	+	+	+	+	+	+	+	+	+	+++
NP3	−	−	−	−	−	−	−	−	−	−	−
1-9	++	−	++	+	+	−	+	+	+	+	−
1-15	±	++	−	+	+	+	−	−	+	+	+++
1-23	++	++	−	++	+	+	−	−	+	+	+++
3-1	++	−	++	+	+	−	+	+	+	+	−
3-2	±	−	++	++	+	−	+	+	+	+	−
4-35	−	+	+	+	+	+	+	+	+	+	ND
17-6	−	++	++	+	+	+	+	+	+	+	+++

ND, not done.
*Frequency of metaphase spreads with relevant chromosome: −, none; ±, <10%; +, 10–30%; ++, >30%.

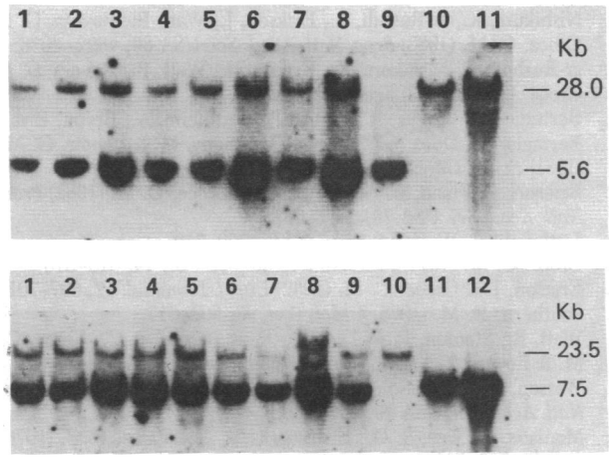

FIG. 4. Southern blotting analysis of NP3–BL2 somatic cell hybrids for the presence of the human c-*myc* gene (*Upper*) and the human c-*mos* gene (*Lower*) after BamHI digestion. (*Upper*) All the hybrids that were analyzed contained the human c-*myc* gene (28.0 kb) (lanes 1–8). NP3 DNA is in lane 9. BL2 DNA is in lane 10 and PAF DNA is in lane 11. (*Lower*) All the hybrids that were analyzed contained the human c-*mos* gene (7.5 kb) (lanes 1–9). NP3 mouse DNA is in lane 10. BL2 DNA is in lane 11 and PAF DNA is in lane 12.

DISCUSSION

The results described in this paper indicate that Burkitt lymphoma cells carrying a t(8;22) translocation contain a C_λ locus translocated to a DNA region 3′ of the c-*myc* oncogene. Thus, it appears that different types of rearrangements, resulting in human immunoglobulin gene sequences being placed either in front of or behind the c-*myc* oncogene, can occur in Burkitt lymphoma. We have previously shown that juxtaposition of either a rearranged or an unrearranged c-*myc* oncogene in front of the C_μ locus results in transcriptional activation of the oncogene (16, 17). The data presented in this paper indicate that translocation of the C_λ locus to a region 3′ of a c-*myc* oncogene in its germ-line configuration can also result in its transcriptional activation. In the same cells, the c-*myc* oncogene on the normal chromosome 8 apparently remains transcriptionally inactive. Therefore, even if the two c-*myc* genes of Burkitt lymphoma cells are structurally identical (not rearranged), only the gene that is located in close proximity to an immunoglobulin locus is activated.

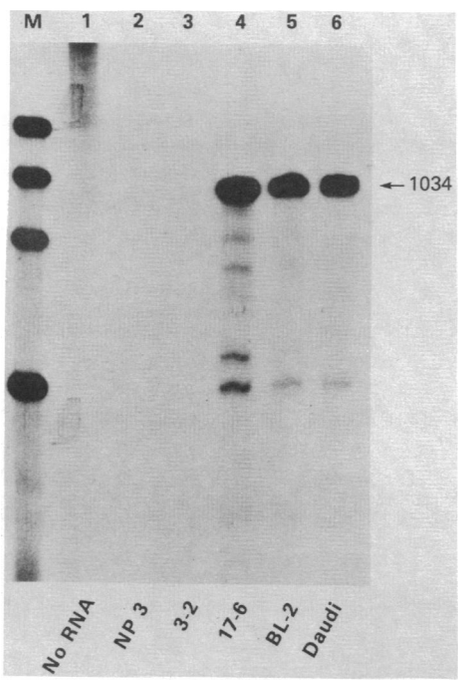

FIG. 5. S1 nuclease protection experiment using a human c-*myc* cDNA probe (Ryc 7.4) specific for the second and third exon of the c-*myc* gene (17). The human *myc* RNA protects a DNA fragment 1,034 nucleotides long (17). Hybrid BL 17-6, which carries the 8q+ chromosome, expresses high levels of c-*myc* transcripts (lane 4). On the other hand, hybrid BL 3-2, which contains the normal 8, the normal 22, and the 22q− chromosomes, does not express human *myc* transcript (lane 3). The parental BL2 Burkitt lymphoma cells and Daudi Burkitt lymphoma cells that carry a t(8;14) chromosome translocation express high levels of human c-*myc* transcripts (lanes 5 and 6, respectively). Lane M, marker DNAs.

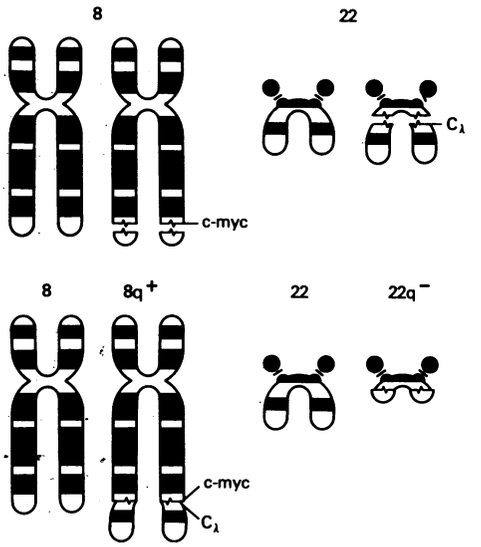

FIG. 6. Diagram of the t(8;22) translocation in Burkitt lymphoma. The C_λ locus moves from its normal location on chromosome 22 to a region distal to the c-*myc* oncogene, which is untranslocated and unrearranged in BL2 cells.

The mechanism of the repression of the c-*myc* oncogenes on the normal chromosome 8 may possibly involve a direct action of the *myc* gene product itself (17). Experiments using a cloned c-*myc* oncogene rearranged with a C_μ gene and transfected into human lymphoblastoid cell lines should establish whether high expression of the transfected oncogene results in the suppression of the normal endogenous c-*myc* gene.

At present the molecular basis of the enhancement of oncogene transcription by genetic elements within immunoglobulin loci is not known. It is reasonable to speculate that, when a c-*myc* oncogene is located in close proximity to an immunoglobulin locus in a B lymphocyte, its transcription is enhanced and it cannot be shut off, resulting in high constitutive levels of c-*myc* expression. This could result in a continuous and irreversible proliferative stimulus for the affected B cells, ultimately leading to neoplasia.

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