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LR1 regulates c-*myc* transcription in B-cell lymphomas

(*MYC* gene/immunoglobulin/chromosome translocation/Burkitt lymphoma)

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ABSTRACT **LR1 is a 106-kDa sequence-specific DNA-binding protein first identified as a potential regulator of immunoglobulin class switch recombination in B lymphocytes. Here we report that LR1 binds to a site 310 nt upstream of the human c-*myc* P1 promoter. Mutation of this site decreases reporter gene expression 5.5-fold in the Burkitt lymphoma line Raji and 3.8-fold in the lymphoma line BJAB. These experiments show that LR1 can function as a transcription factor and identify it as a cell type-specific activator of c-*myc* expression. There are multiple matches to the LR1 recognition consensus at the immunoglobulin heavy-chain locus and at c-*myc*, which further suggests that LR1 may play a dual role, facilitating c-*myc* translocation as well as regulating c-*myc* transcription.**

Deregulated expression of the human c-*myc* gene (*MYC*) characterizes many B-cell lymphomas. In some cases gene amplification accounts for deregulation, but in one kind of human tumor, Burkitt lymphoma, deregulation results from translocation of c-*myc* to one of the immunoglobulin loci, usually the heavy-chain locus (reviewed in refs. 1–7). Translocation breakpoints involve the diversity (D)- or joining (J)-region sequences that participate in variable segment rearrangement or the switch (S)-region sequences that are critical for heavy-chain class switch recombination. This suggests that translocation depends on the recombination mechanisms that are activated during normal B-cell development and that DNA-binding proteins which regulate recombination in developing B cells might also function at c-*myc*.

LR1 is a sequence-specific DNA-binding protein identified as a potential regulator of isotype switch recombination in activated B lymphocytes (8–10). LR1 binds sites in the heavy-chain intron enhancer (E_μ) and in the G-rich S regions. In the S regions the LR1 consensus binding sequence is highly reiterated, with computer search revealing 15–25 potential LR1 binding sites per kilobase of S-region DNA. Bacterial lipopolysaccharide induces LR1 activity in resting primary B cells with kinetics that parallel switch recombination, and dephosphorylation inactivates LR1 DNA-binding activity *in vitro* (8), consistent with regulation by a protein kinase during B-cell activation. LR1 activity is also present in B-cell lines representing recombinationally active developmental stages, including transformed pre-B and B cells.

The possibility that factors that activate B-cell-specific recombination might play a role in c-*myc* translocation led us to ask whether there are LR1 sites within the c-*myc* locus. A search of the 8-kb region that includes the human c-*myc* gene and 2.3 kb of 5′ flanking sequence identified >30 matches to the LR1 binding consensus. One of these, which spans positions −319 to −309 relative to the c-*myc* P1 promoter, is in a region that contains recognition elements for factors known to regulate c-*myc* transcription in other cell types (11–19). Here we show that LR1 binds to this site and that mutation of the site results in a 5.5-fold decrease in reporter gene expression in the Burkitt lymphoma line Raji and a 3.8-fold decrease in reporter gene expression in the B-cell lymphoma line BJAB. These experiments identify LR1 as a cell type-specific regulator of c-*myc* expression and suggest that, like the prokaryotic factor IHF (20), LR1 may play a dual role in both transcription and recombination. The existence of multiple sites for LR1 at both c-*myc* and the heavy-chain locus and the observation that LR1–LR1 interactions occur readily *in vitro* suggest that LR1 bound at the immunoglobulin locus may interact with LR1 bound at c-*myc* to facilitate c-*myc* translocation in recombinationally activated B cells.

MATERIALS AND METHODS

Cell Culture and Nuclear Extract Preparation. Cells were maintained at 37°C in a 5% CO_2 atmosphere in RPMI 1640 medium supplemented with 10% fetal bovine serum, 20 μM 2-mercaptoethanol, penicillin (50 units/ml), streptomycin sulfate (50 μg/ml), and 200 μM L-glutamine. Nuclear extracts were prepared (21) in buffers supplemented with aprotinin, leupeptin, pepstatin A, and benzamidine at 10–20 μg/ml. Protein concentrations were determined by Bradford microassay (Bio-Rad).

DNA Labeling. Labeled duplexes for binding assays were generated by annealing complementary synthetic oligonucleotides and filling recessed ends with the Klenow fragment of DNA polymerase I (8). Specific activity of labeled DNA was 1–2 × 10^8 cpm/μg. The human c-*myc* −310 LR1 binding-site duplex carries sequences from the region spanning −325 to −295 upstream of the c-*myc* P1 promoter; synthetic oligodeoxynucleotide sequences were 5′-GATCCTCACAGGACAAGGATGCGGTTTGTCAAACAA-3′ (top strand) and 5′-GATCTTGTTTGACAAACCGCATCCTTGTCCTGTGAG-3′ (bottom strand). The B2* mutant duplex synthetic oligodeoxynucleotide sequences were 5′-TCGACTCAGATCTACCTTCTGCGGTTTGTCAAAC-3′ (top strand) and 5′-TCGAGTTTGACAAACCGCAGAAGGTAGATCTGAG-3′ (bottom strand). The synthetic duplex 36-mer containing the $S_{\gamma 1}$ LR1 binding site has been described (8).

Gel Mobility-Shift Assays and UV Crosslinking. Gel mobility-shift assays with crude extracts were performed as described (8) in 10-μl reaction mixtures containing 0.4–1 μg of protein, 15–60 pg of labeled DNA, and poly[d(I-C)] at 800-fold mass excess over labeled fragment, in 10 mM Hepes, pH 7.5/150 mm KCl, 0.1% Nonidet P-40 (Calbiochem)/2% (wt/vol) polyvinyl alcohol/5% (vol/vol) glycerol. Poly[d(I·C)] was reduced 100-fold in reactions with purified LR1 (10). After incubation for 20 min at 25°C, complexes were resolved by electrophoresis at 4°C in a nondenaturing 5% polyacryl-

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Abbreviations: CAT, chloramphenicol acetyltransferase; EBV, Epstein–Barr virus; S region; switch region.

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amide gel in 90 mM Tris borate, pH 8.3/2.5 mM EDTA. UV crosslinking was performed as described (9).

DNase I Footprinting. For footprinting, either the top- or the bottom-strand oligonucleotide was 5′ end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase (New England Biolabs) prior to duplex formation. Labeled duplex (10^6 cpm) was incubated with 14 μg of Raji cell nuclear extract, free and bound DNAs were resolved in a nondenaturing 5% polyacrylamide gel, and the gel bands were excised after autoradiography and incubated at room temperature for 1 hr with DNase I (1.5 μg/ml; Worthington) in 20 μl of 10 mM Tris·HCl, pH 8.0/0.05% bovine serum albumin/2 mM dithiothreitol. DNase I digestion was initiated by addition of divalent cations (12 μl of 50 mM $MgCl_2$/50 mM $CaCl_2$), allowed to proceed for 4 min, and stopped by addition of 20 μl of 0.5 M EDTA and 20 μl of 1% SDS. DNA was eluted from the gel slices overnight in 500 μl of 0.5 M ammonium acetate/10 mM Tris·HCl, pH 7.5/1 mM EDTA/0.1% SDS, extracted with phenol/chloroform, and precipitated with ethanol. Equal cpm of bound and free DNA were resolved in an 8 M urea/20% polyacrylamide gel and visualized by autoradiography.

Plasmids and Mutagenesis. Plasmids pCAT-350 and pCAT-290 were the generous gift of J. Lang (ref. 22, where these plasmids are referred to as p238 and p240). In pCAT-350, c-*myc* sequence from −350 nt upstream of the P1 promoter through a site at +511 in c-*myc* exon 1 drives expression of the chloramphenicol acetyltransferase (CAT) gene; pCAT-290 differs in that c-*myc* sequence from −350 through −290 is deleted. Plasmid pCAT-350B2* was generated by PCR-mediated site-directed mutagenesis of pCAT-350. The mutagenic primer, 5′-CCCTTCAAGCTTGGATGATTTATACTCAGATCTACCTTCTGCGGT-3′, was designed to replace the LR1 site with a *Bgl* II site (underlined). The second primer, 5′-GCAACTGACTGAAATGCCTC-3′, was complementary to the CAT coding sequence in the region 86 nt downstream from the ATG start site for CAT mRNA translation. PCR with the mutagenic primer was carried out with pCAT-350 as template for 5 cycles with annealing at 37°C followed by 25 cycles with annealing at 52°C. The PCR product was digested with *Hin*dIII and *Bam*HI and inserted into the *Hin*dIII/*Bam*HI-digested pCAT vector to produce pCAT-350B2*. Mutant clones were identified by digestion with *Bgl* II and sequenced throughout the PCR-amplified region to show that no extraneous mutations had been introduced during amplification.

Transfections and CAT Assays. Transfections were performed by electroporation of 10^7 cells with 10 μg of superhelical DNA using a Bio-Rad Gene Pulser. Raji cells were electroporated at 350 V and 960 μF, and BJAB cells at 300 V and 960 μF. Extracts were prepared 48 hr after transfection (23) and protein concentration was determined by Bradford microassay. CAT assays were carried out in 75-μl reaction mixtures containing 75 μg of protein, 4 μg of acetyl-coenzyme A (Pharmacia), and 0.04 μCi of [^{14}C]chloramphenicol (Amersham; 1 μCi = 37 kBq) in 0.25 M Tris·HCl (pH 7.6), for 1.5 hr at 37°C. After extraction with ethyl acetate, substrate and products were resolved by chromatography in chloroform/methanol, 95:5 (vol/vol), on a thin-layer silica gel plate (Whatman) and acetylation was quantified with a PhosphorImager (Molecular Dynamics). Transfections were carried out in triplicate, and each transfection was repeated multiple times, as described in the legend to Fig. 4. Three preparations of each plasmid were tested, with essentially identical results.

RESULTS

LR1 Binds Near the Promoter of c-*myc*. To determine whether LR1 might function at c-*myc*, we first carried out a computer search for LR1 sites in the 8-kb region that includes c-*myc* and 2.3 kb of upstream DNA (GenBank HUMMYCC). A search that allowed up to two mismatches from the 9-bp consensus found 37 nonoverlapping potential binding sites, 24 in forward and 13 in reverse orientation, as shown in Fig. 1. If DNA is random in sequence, seven specified bases should occur only once per 8 kb. The number of potential LR1 sites at c-*myc* therefore considerably exceeds the number of sites expected by chance, although it does not approach the 15–25 sites per kilobase evident when the same search was carried out on S-region DNA. There does not appear to be an obvious correlation between locations of potential LR1 sites and junction sites for translocation, which cluster just upstream of exon 1 and within this exon and the adjacent intron (see, for example, refs. 3 and 24).

One of the sites identified in the search was at 319 to 309 nt upstream of the P1 promoter and matched the consensus derived from four previously tested LR1 binding sites (8, 10) at eight of nine positions.

LR1 consensus	GGNCNAGGCTG
c-*myc* −319 to −309	—A—A———A—

Complex promoters like that of c-*myc* depend on interactions among the variety of positive and negative transcriptional regulators active in a given cell, and in other cell types regulatory factors have been shown to bind near the −319 to −309 site (11–19). We therefore proceeded to test binding at this site. We assayed extracts of several human hematopoietic cell lines exhibiting deregulated c-*myc* expression for activities that bind the $S_{\gamma 1}$ LR1 site or the c-*myc* −319 to −309 site. Raji and Jiyoye are Epstein–Barr virus (EBV)-transformed Burkitt lymphoma lines in which c-*myc* has undergone translocation to the immunoglobulin heavy-chain locus, BJAB is an EBV-negative B-cell lymphoma with an amplified c-*myc* allele, and HL-60 is a promyelocytic leukemia cell line in which c-*myc* is amplified. Fig. 2 shows that nuclear extracts from all four of these cell lines contain a factor(s) that binds to the $S_{\gamma 1}$ LR1 duplex site and to a synthetic duplex oligonucleotide carrying the human c-*myc* sequence from −325 to −295 (referred to as the c-*myc* −310 duplex). Furthermore, both an $S_{\gamma 1}$ LR1 site duplex and a c-*myc* −310 duplex compete for binding with a fragment spanning the −350 to −225 region of the P1 promoter, while an Oct-site duplex or a c-*myc* −310 B2* mutant duplex do not compete (data not shown).

In some lanes in Fig. 2, a discrete complex, complex II, is apparent that migrates more slowly than the abundant LR1–DNA complex. Complex II is evident in assays both with crude extracts and with highly (14,000-fold) purified LR1, its formation depends upon protein concentration even with highly purified LR1 preparations, and UV crosslinking and label transfer show that complex II contains the same DNA binding species as complex I (L. A. Hanakahi and N.M., unpublished work). We therefore suspect that this complex results from LR1–LR1 interactions; similar behav-

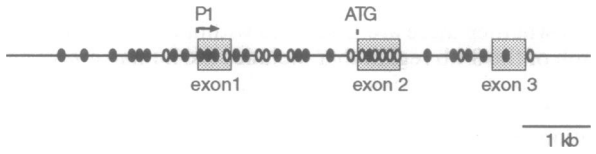


FIG. 1. Matches to the LR1 consensus in the human c-*myc* gene. Computer search of GenBank HUMMYCC identified 37 nonoverlapping matches to the sequence GGNCNAGGCTG, in a search that allowed up to two mismatches. Filled ovals identify sites in forward orientation, and open ovals identify sites in reverse orientation. The figure shows the three c-*myc* exons, the start site for transcription from the P1 promoter, and the ATG start site for translation.

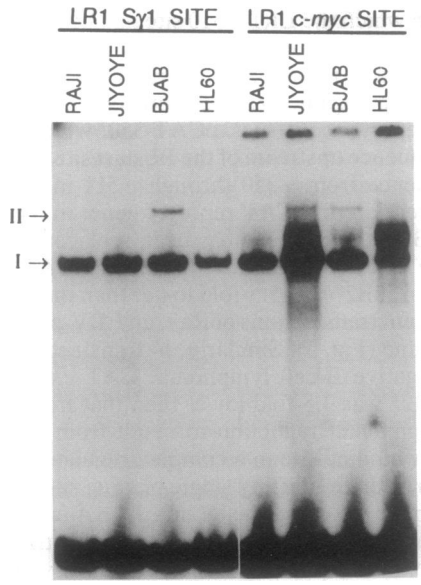


FIG. 2. Gel mobility-shift assays of LR1 $S_{\gamma 1}$- and c-*myc*-site binding activities in human hematopoietic cell lines. ^{32}P-labeled synthetic duplex oligonucleotides containing the $S_{\gamma 1}$ or c-*myc* −310 LR1 binding sites were incubated with 1 μg of nuclear extract from the human Burkitt lymphoma cell lines Raji and Jiyoye, the B-cell lymphoma line BJAB, and the promyelocytic leukemia cell line HL-60. Arrows denote two complexes formed by LR1; complex II appears to result from LR1–LR1 interaction (see text). In binding assays with 1 μg of crude extract, as shown here, the Jiyoye and HL-60 lanes are clearly overloaded for the c-*myc* −310 binding activity. A broad and rather smeared band running just behind LR1 is evident in the assays with these extracts using the c-*myc* but not the $S_{\gamma 1}$ site; this complex is also evident in assays with HL-60 extracts run at lower protein concentration, but the origin of this band is not clear.

ior in gel shift assays has been described for transcription factor Sp1 (25).

The Protein That Binds the c-*myc* −310 Site is LR1. To confirm that the protein that bound to the c-*myc* −310 duplex was in fact LR1, we assayed binding of highly purified LR1 (10) to the $S_{\gamma 1}$ and c-*myc* sites. Purified LR1 bound comparably to the $S_{\gamma 1}$ and c-*myc* −310 duplex oligonucleotides (Fig. 3*A*). Other experiments have shown that this purified protein does not bind either an Sp1 (26) or a BSAP (27) duplex site under these binding conditions (ref. 10 and data not shown). We also compared the abilities of Raji and BJAB crude nuclear extracts and purified LR1 to bind the c-*myc* −310 duplex and the B2* duplex, in which the LR1 consensus was mutated to produce a *Bgl* II site. Binding of purified LR1 to the c-*myc* duplex produced a shift identical in mobility to that observed upon binding by Raji and BJAB crude extracts, and mutation of sequences within the consensus abolished binding (Fig. 3*B*). The faint band apparent with purified protein appears to reflect nonspecific affinity for DNA, as it vanished when higher concentrations of nonspecific competitor were included in the binding reaction mixtures (data not shown).

UV crosslinking established that the $S_{\gamma 1}$ and c-*myc* DNA-binding activities in crude nuclear extracts of Raji, HL-60, BJAB, and Jiyoye had the same molecular weight as LR1. ^{32}P-labeled, 5-bromodeoxyuridine-substituted oligonucleotides corresponding to the $S_{\gamma 1}$ and c-*myc* LR1 binding sites were incubated with crude nuclear extracts and the complexes were resolved by gel mobility shift. The shifted complexes were then UV crosslinked in the gel, excised from the gel as intact bands, and polymerized into the stacking gel of an SDS/polyacrylamide gel as described (9). In all cases, label was transferred from the duplex oligonucleotide to a single polypeptide of 106 kDa (Fig. 3*C*).

Binding Is Centered at the LR1 Consensus and Creates a Region of DNase I Hypersensitivity. The c-*myc* −310 duplex oligonucleotide with either the top or the bottom strand 5′ end-labeled was incubated with Raji cell nuclear extract, and free and bound DNAs were resolved by nondenaturing gel electrophoresis. After DNase I treatment in the gel, DNA was eluted and electrophoresed in an 8 M urea/polyacryl-

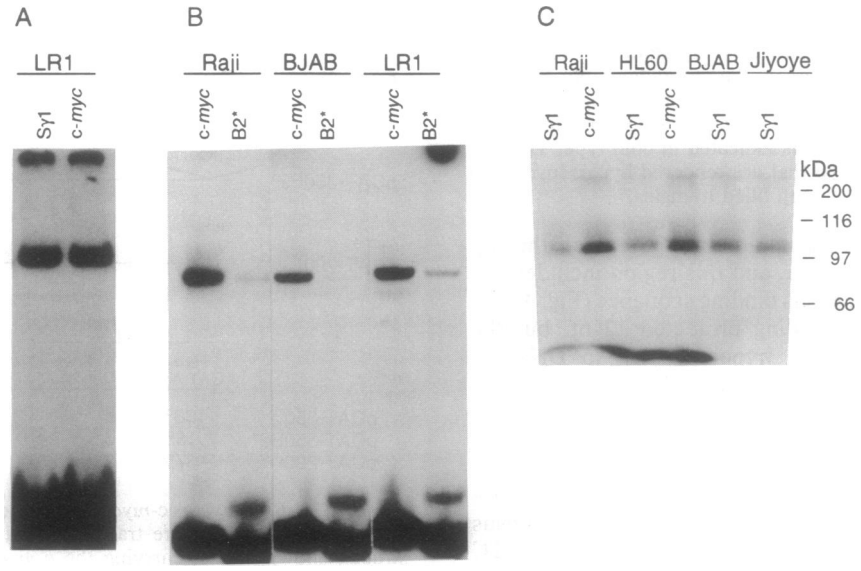


FIG. 3. The protein binding to the c-*myc* −310 site is LR1. (*A*) Gel mobility-shift assay of binding of purified LR1 to the c-*myc* −310 site. ^{32}P-labeled synthetic duplex oligonucleotides containing either the $S_{\gamma 1}$ or the c-*myc* −310 LR1 binding site were incubated with LR1 that was 14,000-fold purified from murine PD31 pre-B cells (10). (*B*) Gel mobility-shift assay of binding to the c-*myc* −310 site and B2* mutant sites. ^{32}P-labeled synthetic duplex oligonucleotides containing either the c-*myc* −310 site or the B2* mutant site were incubated with crude Raji or BJAB nuclear extract or with LR1 that was 1000-fold purified from Raji cells. (*C*) UV crosslinking and label transfer. ^{32}P-labeled 5-bromodeoxyuridine substituted $S_{\gamma 1}$ and c-*myc* LR1 binding-site oligonucleotides were incubated with nuclear extract from Raji, HL-60, BJAB, or Jiyoye cells, and complexes were resolved by mobility-shift assay. After crosslinking *in situ* by UV irradiation (9), labeled polypeptides were resolved by electrophoresis in an SDS/7.5% polyacrylamide gel. Positions of prestained molecular size standards (Bio-Rad) are shown at right. The material at the bottom of the autoradiogram is free DNA, which runs with the dye front.

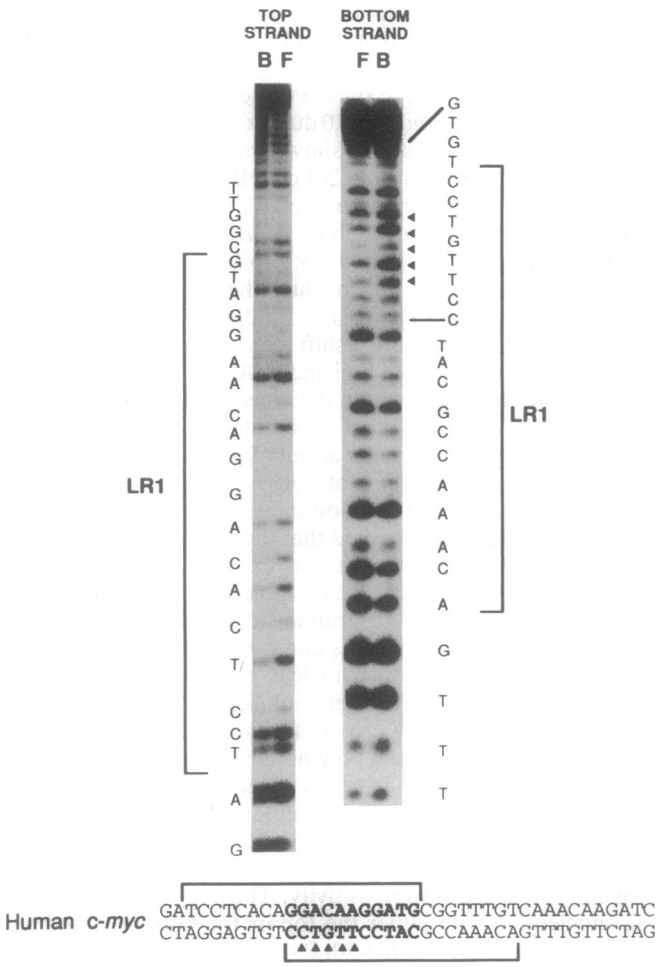
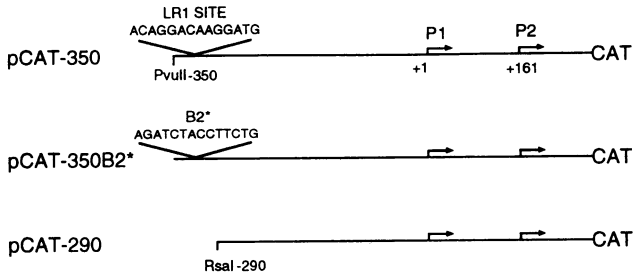


FIG. 4. DNase I footprinting of the c-*myc* LR1 binding site. Synthetic duplexes containing the c-*myc* −310 site, 5′ end-labeled on the top or bottom strand, were incubated with Raji nuclear extract, and bound (B) and free (F) DNAs were separated in a nondenaturing 5% polyacrylamide gel, treated with DNase I *in situ*, eluted from the gel, and resolved in an 8 M urea/20% polyacrylamide gel. The results of the footprinting are shown below the autoradiograph. Brackets above and below the sequence denote the minimum region affected by protein binding. The match to the consensus LR1 binding site at the center of the binding region is indicated in bold type. The five positions on the bottom strand that are rendered hypersensitive to DNase I cleavage are marked with filled triangles.

amide gel. LR1 protected a minimum of 17 nt on the top strand from DNase I cleavage, and this region included the 11-base match to the consensus binding sequence (Fig. 4). On the bottom strand, LR1 binding protected 12 nt, but the adjacent 5 nt were rendered hypersensitive to DNase I cleavage. While it is not uncommon to observe one or two hypersensitive positions in a DNase I footprint, hypersensitivity at five contiguous positions correlates with significant changes in DNA conformation upon protein binding (see, for example, ref. 28). The binding regions on the two strands are staggered and center around the match to the LR1 consensus sequence, GGNCNAGGCTG. The hypersensitive TTGTC sequence on the bottom strand is within the DNA binding consensus and is present as a palindrome with 9-bp spacing in the c-*myc* site. The significance of this palindromic region is not clear, however, and palindromic sequences do not characterize the other LR1 sites analyzed (ref. 8 and unpublished data).

LR1 Activates c-*myc* Transcription in Raji and BJAB Cells. To determine whether LR1 binding could regulate c-*myc* transcription, we assayed expression of a c-*myc* reporter construct in which the LR1 −310 site had been mutated. The c-*myc* promoter is complex and contains sites for other regulatory factors as well as potential LR1 sites throughout the 5′ flanking region. We therefore chose for mutational analysis a reporter construct, pCAT-350, which carries only 350 nt of sequence upstream of the P1 start site. In pCAT-350, c-*myc* sequence from −350 through +511 in c-*myc* exon 1 drives expression of a CAT reporter gene; in its derivative, pCAT-350B2*, the LR1 site has been replaced with the B2* mutant site (see *Materials and Methods*). CAT expression from pCAT-350B2* was 5.5-fold lower than that from pCAT-350 in transient transfections of Raji, an EBV-positive Burkitt lymphoma line (Fig. 5). Similarly, in transfections of BJAB, an EBV-negative B-cell lymphoma, CAT expression from pCAT-350B2* was 3.8-fold lower than that from pCAT-350. This is a significant reduction to result from mutation of a single factor binding site in a complex regulatory region; for comparison, mutation of any single binding site in the heavy-chain enhancer typically results in a 2- to 4-fold decrease in enhancer function (29). These results demonstrate that LR1 can activate transcription from the human c-*myc* promoter in two different B-cell lymphoma lines.

Deletion of sequence from −353 to −293 stimulates expression 10-fold in transfection of fibroblasts, which do not contain LR1 activity; the net repression associated with this region in fibroblasts appears to be mediated by a Fos-related negative regulatory factor that binds sequences from −343 to −318 (13–15). To ask whether the −350 to −290 region conferred positive or negative regulation in lymphomas, we transfected a construct, pCAT-290, in which sequence upstream of −290 is deleted to generate a construct which lacks both the LR1 site and the potential upstream binding site. CAT expression from pCAT-290 was 2.7-fold lower than from pCAT-350 in Raji cells, and 1.8-fold lower in BJAB (Fig. 5). This 60-nt deletion thus decreases reporter gene expression less than does mutation of the LR1 site. This suggests that a negative regulatory factor may function in the −350 to −290 region in lymphoma lines. However, the overall regulation conferred by the −350 to −290 region in lymphomas is



	Raji		BJAB	
	Relative CAT Activity	Fold Decrease	Relative CAT Activity	Fold Decrease
pCAT-350	100%	—	100%	—
pCAT-350B2*	18%	5.5 ± 1.4	26%	3.8 ± 0.9
pCAT-290	37%	2.7 ± 0.5	55%	1.8 ± 0.3

FIG. 5. LR1 activates c-*myc* transcription in Raji and BJAB cells. Raji and BJAB cells were transfected with CAT expression constructs, shown above, carrying the c-*myc* promoter region (from −350 or −290 through +511) with the LR1 site intact (pCAT-350), mutated (pCAT-350B2*), or deleted (pCAT-290). Data are from five transfections of Raji and BJAB cells with pCAT-350B2*, seven transfections of Raji cells with pCAT-290, and three transfections of BJAB cells with pCAT-290. All transfections were carried out in triplicate. Three preparations of each plasmid were tested, with essentially identical results. CAT expression was assayed 2 days after transfection, and acetylation was quantitated by PhosphorImager. Expression was normalized to the pCAT-350 construct. Fold decrease is shown with standard error.

activation, in contrast to the repression associated with this region in fibroblasts. Further analysis of the −350 to −290 region will show whether the same factor identified in fibroblasts is active in lymphomas and whether activation by LR1 overrides repression at the upstream site.

DISCUSSION

We have shown that LR1 can function as a transcription factor, binding to a site 310 nt upstream of the P1 promoter to activate c-*myc* expression in the B-cell lymphoma lines Raji and BJAB. LR1 was originally identified as a potential regulator of heavy-chain isotype switch recombination in activated B cells (8), and the observation that LR1 can also regulate transcription suggests that LR1, like the prokaryotic factor IHF (20), has a dual function in both transcription and recombination. In addition to LR1, other positive and negative transcriptional regulators have been shown to bind to sites within the c-*myc* 5′ flanking region (11–19), but to our knowledge LR1 is the first cell type-specific factor shown to regulate c-*myc* in B-cell lymphomas.

Translocation appears to release c-*myc* from controls that negatively regulate both transcriptional initiation and elongation, but the heterogeneity of c-*myc* translocations has made it difficult to understand what elements activate transcription of the translocated gene (1–8). Deletion and substitution mutations are frequently evident in translocated c-*myc* alleles, but these mutations do not account for deregulated expression (30, 31). While c-*myc* and the heavy-chain intron enhancer, E_μ, are juxtaposed in some t(8;14) translocations, in others E_μ is translocated to the former site of c-*myc* on chromosome 8. Furthermore, while translocation breakpoints may be as much as several hundred kilobases upstream of c-*myc* (32), in some cases breakpoints are within c-*myc* and sever the coding region from its own promoters and upstream regulatory sequences. The observation that LR1 can regulate c-*myc* expression may help to explain how at least some of these translocations contribute to c-*myc* deregulation. There are multiple LR1 binding sites in each of the heavy-chain S regions, and LR1 bound at these sites might activate c-*myc* in the absence of E_μ or c-*myc* upstream elements.

Our data raise the possibility that LR1, in addition to its normal role in switch recombination, may also facilitate the c-*myc* translocation events that contribute to many B-cell malignancies. Translocation and switch recombination occur in the same developmental stage of the same cell type, and translocation breakpoints, like switch recombination junctions (33), are heterogeneous with respect to both site and junction sequence. It is therefore plausible that the two processes share similar molecular mechanisms. LR1 has multiple binding sites in c-*myc* and in the heavy-chain locus. LR1–LR1 interactions occur readily *in vitro*. If such interactions also occur *in vivo*, then LR1 bound at the immunoglobulin locus may interact with LR1 bound at the c-*myc* locus to stimulate or target aberrant recombination events, thereby extending the normal role of LR1 in recombination to a pathological one.

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1. Cory, S. (1986) *Adv. Cancer Res.* **47**, 189–234.
2. Croce, C. M. & Nowell, P. C. (1986) *Adv. Immunol.* **38**, 245–274.
3. Kelly, K. & Siebenlist, U. (1986) *Annu. Rev. Immunol.* **4**, 317–338.
4. Haluska, F. G., Tsujimoto, Y. & Croce, C. M. (1987) *Annu. Rev. Genet.* **21**, 321–345.
5. Waldmann, T. A. (1990) *Adv. Immunol.* **40**, 247–321.
6. Magrath, I. (1990) *Adv. Cancer Res.* **55**, 133–270.
7. Spencer, C. A. & Groudine, M. (1991) *Adv. Cancer Res.* **56**, 1–48.
8. Williams, M. & Maizels, N. (1991) *Genes Dev.* **5**, 2353–2361.
9. Williams, M., Brys, A., Weiner, A. M. & Maizels, N. (1992) *Nucleic Acids Res.* **20**, 4935–4936.
10. Williams, M., Hanakahi, L. A. & Maizels, N. (1993) *J. Biol. Chem.* **268**, 13731–13737.
11. Kakkis, E. & Calame, K. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7031–7035.
12. Kakkis, E., Riggs, K. J., Gillespie, W. & Calame, K. (1989) *Nature (London)* **339**, 718–721.
13. Hay, N., Bishop, J. M. & Levens, D. (1987) *Genes Dev.* **1**, 659–671.
14. Hay, N., Takimoto, M. & Bishop, J. M. (1989) *Genes Dev.* **3**, 293–303.
15. Takimoto, M., Quinn, J. P., Farina, A. R., Staudt, L. M. & Levens, D. (1989) *J. Biol. Chem.* **264**, 8992–8999.
16. Postel, E. H., Mango, S. E. & Flint, S. J. (1989) *Mol. Cell. Biol.* **9**, 5123–5133.
17. Thalmeier, K., Synovzik, H., Mertz, R., Winnacker, E.-L. & Lipp, M. (1989) *Genes Dev.* **3**, 527–536.
18. Duyao, M. P., Buckler, A. J. & Sonenshein, G. E. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4727–4731.
19. Riggs, K. J., Merrell, K. T., Wilson, G. & Calame, K. (1991) *Mol. Cell. Biol.* **11**, 1765–1769.
20. Friedman, D. (1988) *Cell* **55**, 545–554.
21. Dignam, J. D., Lebovitz, R. M. & Roeder, R. G. (1983) *Nucleic Acids Res.* **11**, 1475–1489.
22. Spandidos, D. A., Zoumpourlis, V. & Lang, J. C. (1991) *Anticancer Res.* **11**, 1339–1342.
23. Gorman, C. M., Moffat, L. F. & Howard, B. (1982) *Mol. Cell. Biol.* **2**, 1044–1051.
24. Pelicci, P.-G., Knowles, D. M. II, Macrath, I. & Dalla-Favera, R. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2984–2988.
25. Pascal, E. & Tjian, R. (1991) *Genes Dev.* **5**, 1646–1656.
26. Ahn, J. K., Gavin, B. J., Kumar, G. & Ward, D. C. (1989) *J. Virol.* **63**, 5425–5439.
27. Barberis, A., Widenhorn, K., Vitelli, L. & Busslinger, M. (1990) *Genes Dev.* **4**, 849–859.
28. Salvo, J. & Grindley, N. D. F. (1988) *EMBO J.* **7**, 3609–3616.
29. Lenardo, M., Pierce, J. W. & Baltimore, D. (1987) *Science* **236**, 1573–1577.
30. Richman, A. & Hayday, A. (1989) *Science* **246**, 494–497.
31. Spencer, C. A., LeStrange, R. C., Novak, U., Hayward, W. S. & Groudine, M. (1990) *Genes Dev.* **4**, 75–88.
32. Joos, S., Falk, M., Lichter, P., Haluska, F. G., Henglein, B., Lenoir, G. M. & Bornkamm, G. W. (1992) *Hum. Mol. Genet.* **8**, 625–632.
33. Dunnick, W., Hertz, G. Z., Scappino, L. & Gritzmacher, C. (1993) *Nucleic Acids Res.* **21**, 365–372.