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Trans-acting elements modulate expression of the human c-*myc* gene in Burkitt lymphoma cells

(transient expression/competition/oncogene)

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ABSTRACT **We have used a competition assay to identify the targets of trans-acting elements that modulate the expression of the human c-*myc* gene (designated *MYC* in human gene nomenclature). For this purpose, a c-*myc* hybrid indicator gene was formed by joining the c-*myc* promoter region, first noncoding exon, and intron to the bacterial gene for chloramphenicol acetyltransferase (CAT). The test assay consisted of cotransfecting the indicator gene with competing fragments of DNA derived from suspected control regions of the c-*myc* gene. Such experiments test the hypothesis that control regions are often targets for the binding of trans-acting regulatory factors that can be diverted to competing fragments of DNA. A negatively acting element will be diverted from the indicator gene, allowing the gene's enhanced expression, whereas a positively acting element will behave oppositely. Control indicator genes driven by non-*myc* promoters assess the specificity of the effect. Using this approach, we find three c-*myc* regions that are capable of enhancing the expression of the indicator gene in competition assays (i.e., putative sites of negative modulation). In addition, we find sequences near the c-*myc* promoters that suppress expression in competition assays (i.e., putative binding sites of positively acting factors). These results, with appropriate controls, suggest the existence of target sites near the c-*myc* gene that specifically modulate its expression both positively and negatively. Their locations fit well with regions damaged or lost in many Burkitt lymphoma and murine plasmacytoma translocations.**

The c-*myc* gene is the cellular homologue of the oncogene v-*myc*, the transforming gene of the avian retrovirus MC29 (1). Activation of its expression has been implicated in the oncogenesis of a variety of neoplasias in different species: B-cell lymphoma in chicken (2, 3); promyelocytic leukemia HL60 (4), APUDoma Colo 320 (5), Burkitt lymphoma (6, 7), and small-cell lung carcinoma (8) in man; plasmacytoma (9–11) and T-cell lymphoma (12) in mouse; and T-cell lymphoma (13) in cat. The activation of the c-*myc* gene (designated *MYC* in human gene nomenclature) in these neoplasms is accomplished through a variety of mechanisms, including nearby integration of a retrovirus, gene amplification, and, in the case of Burkitt lymphoma and murine plasmacytoma, translocation of one of the c-*myc* alleles into one of the immunoglobulin loci (for review, see ref. 14). The primary defect is thought to involve deregulation of the c-*myc* gene, although in the case of Burkitt lymphoma and murine plasmacytoma, the amount of c-myc mRNA (15) and protein (16) is not significantly greater than that seen in control B cells. However, most of the mRNA arises from the translocated rather than the germ-line c-*myc* allele in transformed cells (15, 17, 40).

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There is substantial evidence that c-*myc* expression is associated with cell growth. When HL60 (18) or erythroleukemic cells (19) are chemically induced to terminally differentiate, c-*myc* expression declines significantly prior to the cessation of cell division. Similarly, quiescent lymphocytes and fibroblasts express very little, if any, c-myc mRNA. When such cells are stimulated with a mitogen and induced to enter the cell cycle, c-*myc* expression increases by 10- to 40-fold within 2 hr (20). There are conflicting reports as to whether c-*myc* induction occurs at the transcriptional level (21) or the posttranscriptional level (22). However, once the cell enters the cell cycle, there appears to be no change in the c-*myc* expression throughout the cycle (23, 24). Therefore, it has been suggested that the c-*myc* product plays a role in the progression of cells from resting to the actively dividing state in the cell cycle. Indeed, primary rat embryo fibroblasts transfected with a highly expressible c-*myc* construct become immortalized under certain conditions (25).

It is reasonable to hypothesize that inappropriate expression of the c-*myc* gene might prevent the cell from entering the resting state. Translocations might result in such expression of c-*myc* by any of several mechanisms including: (*i*) deletion or mutation of regulatory region(s); (*ii*) translocation next to enhancer-like sequences in one of the immunoglobulin loci; or (*iii*) translocation into a chromatin structure that is more conducive to c-*myc* expression in B cells (14). Understanding the mechanism by which this gene is activated will undoubtedly require an understanding of how the c-*myc* gene is normally regulated and an identification of the cis- and trans-acting elements that mediate its control. Toward this end, we have carried out a series of transient transfections utilizing the CAT assay system (26). We present evidence that suggests that the germ-line c-*myc* gene is regulated by c-*myc*-specific positive and negative regulatory factors that bind to multiple sites near and within the c-*myc* gene. The putative positive regulatory factors bind to two distinct regions near the promoters. The putative negative regulatory factor(s) binds to an upstream site as well as to sites within the c-*myc* gene.

MATERIALS AND METHODS

Tissue Culture and DNA-Mediated Transfer. The Ly65 Burkitt lymphoma cells were grown in RPMI 1640 medium with 15% fetal calf serum, 1% penicillin and streptomycin, and 28 μM 2-mercaptoethanol and were split approximately 1:10 every 3 days. The cells were grown in 93% air/7% CO_2 at 37°C.

Abbreviations: CAT, chloramphenicol acetyltransferase; bp, base pair(s); SV40, simian virus 40.

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The Ly65 cells were transfected by using the electroporation technique, which has been described (27). Briefly, a confluent culture of cells was split 1:3 or 1:4 the day before the transfection. Immediately before the transfection, the cells were washed once with sterilized phosphate-buffered saline (137 mM NaCl/2.7 mM KCl/8.1 mM Na_2HPO_4/1.5 mM KH_2PO_4, pH 7.3). The cells were then resuspended in 0.5 ml of phosphate-buffered saline at 2×10^7 cells per ml. The cells were mixed with DNA in the electroporation chamber and left on ice for 10–20 min, and then a 2000-V pulse was applied. The cells were then resuspended in 35 ml of fresh medium.

Assays for CAT Gene Expression. Approximately 48 hr after the transfection, the cells were washed once with cold phosphate-buffered saline and resuspended in 0.1 ml of 250 mM Tris·HCl (pH 7.6). CAT activity was assayed as described by Gorman *et al.* (26) except that the acetyl CoA concentration was increased to 5 mM. The CAT reaction was usually terminated with ethyl acetate after 2 hr and 4 hr to obtain the acetylation rate as a function of time. Quantitation was performed as described by Gorman *et al.* (26).

RESULTS

In order to monitor the activity of the c-*myc* promoters using the bacterial CAT assay, we constructed the human c-*myc*–CAT gene hybrid and mutants as described in the legend to Fig. 1. The c-*myc*/CAT gene plasmid Z2 carries the c-*myc* promoter, first exon, first intron, and 6.8 kilobases of upstream c-*myc* sequences. It properly initiates transcripts when expressed in BALB/c 3T3 cells by stable transfection (data not shown). To localize potential upstream regulatory sequences, we tested a series of 5′ deletion mutants derived from the wild-type c-*myc*/CAT gene Z2 (Fig. 1). These deletions relative to the first transcription initiation site are as follows: del HC, −2325 to −1250; del HR, −2325 to −293; del HS, −2325 to −101; del CR, −1250 to −293; del CS, −1250 to −101; and del PS, −350 to −101. The Burkitt lymphoma cell line Ly65 was transfected by electroporation with 10 μg

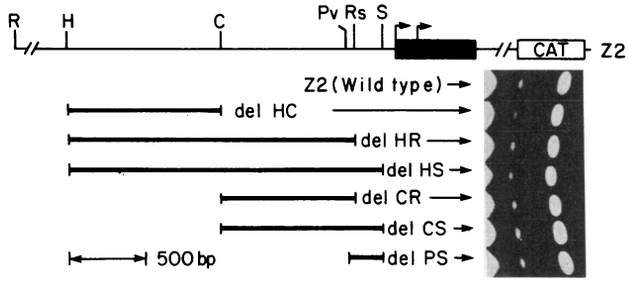

FIG. 1. Structure of the c-*myc*/CAT gene hybrid and the CAT assay of the upstream deletion mutants. The upstream regions indicated by the heavy lines were deleted from the wild-type c-*myc*/CAT gene, Z2. Ten micrograms of Z2 or the deletion-containing derivatives were transfected into Ly65 cells by electroporation. The cells were harvested after 48 hr at which time the CAT activity was assayed. The c-*myc*/CAT gene was constructed from the 12.7-kilobase *Eco*RI–*Eco*RI fragment containing the human germ-line c-*myc* gene. This fragment was cut at the *Bst*EII site in the second exon and digested with BAL-31 enzyme so that only 9 base pairs (bp) of the second exon remained, as verified by sequencing. This digestion deleted the translation initiation codon ATG of the c-*myc* gene but retained the splice acceptor site of the second exon. The CAT gene segment from pCM4 (41) was then ligated to the *Bam*HI linker previously introduced just downstream of the splice acceptor site. The c-*myc*/CAT gene Z2 contains 6.8 kilobases of the 5′ region up to the *Eco*RI site and is cloned into the pBR327 vector. The black rectangle represents the first exon, and the two bent arrows, the mRNA initiation sites. R, *Eco*RI; H, *Hin*dIII; C, *Cla* I; Pv, *Pvu* II; Rs, *Rsa* I; S, *Sma* I.

of either Z2 DNA or the mutant c-*myc*/CAT gene DNA. After approximately 48 hr, the cells were harvested and assayed for CAT activity. As shown in Fig. 1, the deletions appear to have no significant effect on the activity of the c-*myc* promoters. Similar results were obtained when these constructs were transfected into L cells or NIH 3T3 cells (data not shown).

In testing for regulatory sequences in the first exon and intron of the c-*myc* gene, one cannot simply delete sequences and assay effects on CAT gene expression. In addition to creating new sequences by juxtapositions, the deletion might alter the posttranscriptional processing of the mRNA. Therefore, we used a competition assay (28) in which the c-*myc*/CAT indicator gene, pZHB2 [very similar to Z2 (Fig. 1), only modified according to the legend to Fig. 2], is cotransfected with an excess of a potential regulatory sequence that might compete for a trans-acting factor(s) that affects transcription. In this case, the indicator gene would be deprived of this factor(s). Thus, if the competing sequence binds a positive regulatory factor, expression of the indicator gene would be reduced. Reduction in expression would be measured in comparison to a control competing sequence that should not bind the c-*myc*-specific factor(s). Conversely, if the competing sequence binds a negative regulatory factor, the expression of the indicator gene would be induced in comparison to the control. We used the plasmid vector pSP65 as the control in all our competition assays.

In order to test the region that includes the first exon and the first intron of the c-*myc* gene, a 2.7-kb *Pst* I–*Pst* I fragment (Fig. 2) was cut at the *Xba* I site, and the two resulting *Pst* I–*Xba* I fragments were subcloned into the pSP65 vector. The subclone pPsX includes the sequence from position −407 to +1180, and the subclone pXPs, from +1180 to +2321. Ly65 Burkitt lymphoma cells were cotransfected with 5 μg of the indicator gene pZHB2 and 50 μg of pSP65 vector alone or subclones pPsX or pXPs. The results of the CAT assay are shown at the top of Fig. 2. The competition assay with subclone pPsX increased the CAT activity by more than 5-fold over the activity measured with vector alone or with the subclone pXPs.

To define more precisely the region that induces the expression of the indicator gene in the competition assay, we

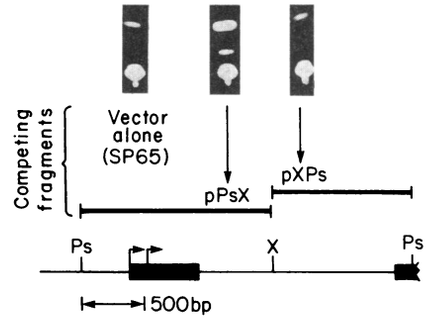

FIG. 2. Effect of competition with DNA fragments derived from the first exon and the intron of the c-*myc* gene on the expression of the c-*myc* promoter-driven indicator gene. The 2.7-kilobase *Pst* I–*Pst* I fragment shown at the bottom of the figure was cut at the *Xba* I site (X) and the two fragments indicated by the heavy lines were cloned into the pSP65 vector. The plasmid pPsX contains the 5′ *Pst* I–*Xba* I fragment, and pXPs, the 3′ *Xba* I–*Pst* I fragment. Five micrograms of the indicator gene, pZHB2 (see below), was cotransfected with 50 μg of pSP65 vector alone, pPsX, or pXPs into Ly65 cells by electroporation. Indicator gene pZHB2 is similar to wild-type c-*myc*/CAT gene Z2 (see the legend to Fig. 1) except that pZHB2 contains only 2.3 kilobases of the 5′ region up to the *Hin*dIII site and is cloned into the pSP65 vector. The CAT assay from this competition assay is shown at the top. Ps, *Pst* I. CAT activity in percentage acetylation per hr was assayed: SP65, 0.4; pPsX, 3.1; pXPs, 0.5.

isolated and cloned into the pSP65 vector the following fragments, which together span most of the first exon and part of the first intron of the c-*myc* gene: pNPv, from position +210 to +510; pNXS-1, from +210 to +1180; pPXS-1, from +510 to +1180; and pSSS, from +942 to +1548 (Fig. 3). Again, the Ly65 Burkitt lymphoma cells were cotransfected with 5 μg of the indicator gene pZHB2 and 50 μg of the vector alone or subclones pNPv, pNXS-1, pPXS-1, or pSSS. The competition assays with pNPv, pNXS-1, pPXS-1, and pSSS all induced the activity of the indicator gene relative to that with the vector alone (Fig. 3*A*).

To demonstrate that induction of the indicator gene with the competitor fragments from the first exon and first intron is c-*myc* specific, we performed the same competition assay with a control indicator gene, pA10BB (Fig. 3*B*). The indicator gene pA10BB is similar to pA10CAT-2 (29), which has

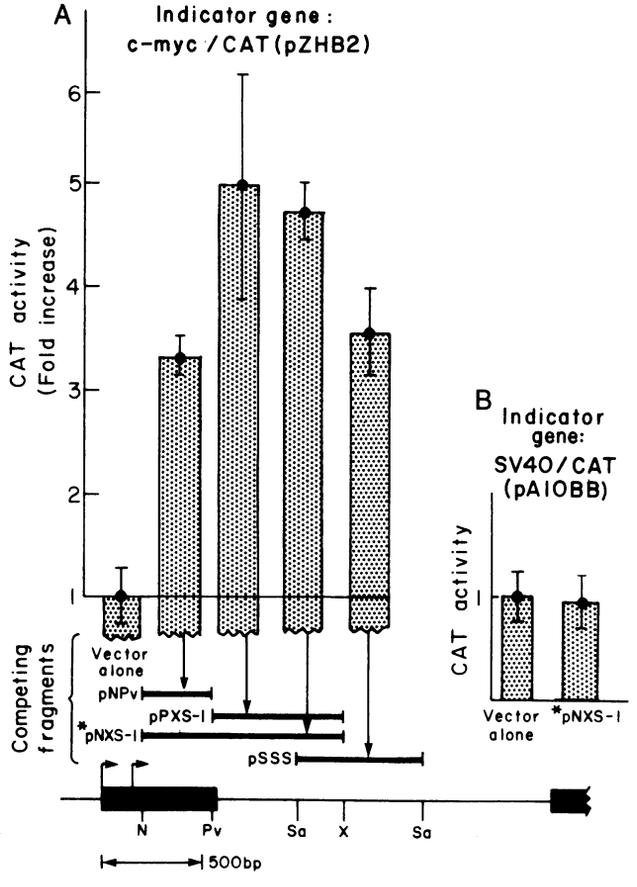

FIG. 3. Assessment of the effect of competition with subfragments derived from the c-*myc* gene on the expression of the c-*myc* promoter-driven indicator gene (Fig. 1). The DNA fragments indicated by the heavy lines were cloned into the SP65 vector and used as competitors in competition assays in Ly65 cells. The CAT activity from the competition assays with various competitors is shown as a fold increase over the activity measured with the vector alone. The map of the c-*myc* gene at the bottom indicates the derivation of these fragments. (*A*) Indicator gene pZHB2 (5 μg) was separately cotransfected with 50 μg of each of the competitors and the pSP65 vector alone. CAT activity in percentage acetylation per hr was assayed: pSP65, 0.4, 0.7; pNpv, 1.7, 1.9; pPXS-1, 2.0, 3.3; pNXS-1, 2.4, 2.6; and pSSS, 1.7, 2.1. (*B*) Indicator gene pA10BB (5 μg; SV40 early promoter without the enhancer driving the CAT gene) (see below) was cotransfected with 50 μg of the pSP65 vector alone or pNXS-1. The asterisk (*) next to pNXS-1 indicates that it was used as a competitor with both indicator genes. The CAT activity in percentage acetylation per hr was assayed: pSP65, 0.5, 0.8; and pNXS-1, 0.4, 0.7. pA10BB was constructed by cloning the *Bgl* II–*Bam*HI fragment of pA10CAT-2 (29) into the pSP65 vector. N, *Nae* I; Pv, *Pvu* II; Sa, *Sac* I; X, *Xba* I.

the enhancerless simian virus 40 (SV40) early promoter driving the CAT gene (SV40/CAT indicator gene) except that it is cloned into the pSP65 vector. The Ly65 Burkitt lymphoma cells were cotransfected with 5 μg of pA10BB and 50 μg of the vector alone or subclone containing pNXS-1. The competition assay with the pNXS-1 subclone had no effect on the expression of the SV40 promoter-driven indicator gene, pA10BB (Fig. 3*B*).

Interestingly, analysis of the upstream deletion mutants of the c-*myc*/CAT gene hybrid (Fig. 1) failed to reveal any potential regulatory regions. From the foregoing, it is clear that this might be due to the putative negative regulatory factor(s) binding to multiple sites in the first exon and intron, thereby masking any effect of the upstream deletions. If there were upstream sequences that also bind to this negative regulatory factor(s), one should be able to utilize these sequences to enhance the activity of the c-*myc*/CAT indicator gene in a competition assay. In order to test this, the Ly65 Burkitt lymphoma cells were cotransfected with 5 μg of the indicator gene, pZHB2, and 50 μg of the vector alone or subclones containing the following fragments: pDSS, from position −1967 to −1500; p5-1, from −1500 to −1259; p5-2, from −1250 to −1054; p5-3, from −1054 to −883; p5-4, from −883 to −608; and p5-5, from −608 to −407 (Fig. 4). The indicator gene was induced only in a competition assay with p5-5 (Fig. 4*A*). When the control indicator gene, pA10BB, was used in a competition assay with either the vector alone

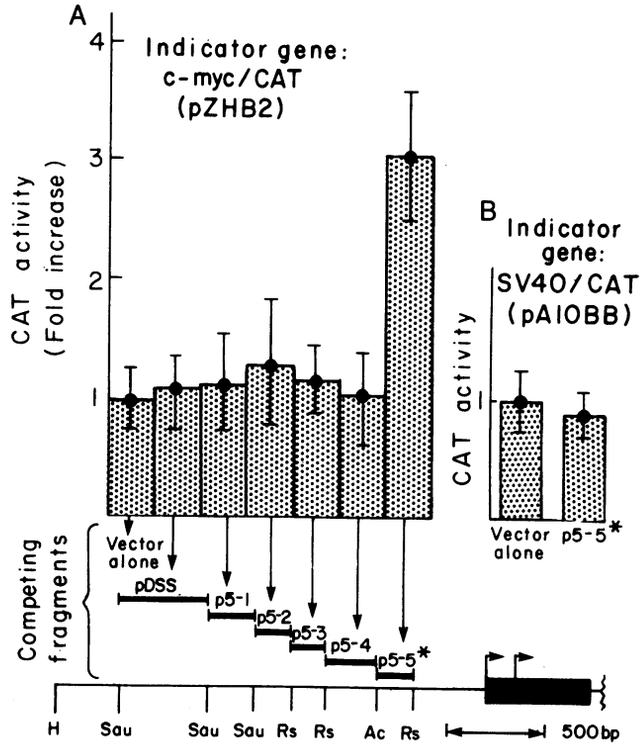

FIG. 4. Assessment of the effect of 5′ c-*myc* fragments on expression of the indicator gene. (*A*) The competition assay was carried out as described in Fig. 3*A* using the competitor fragments indicated by the heavy lines above the map of the c-*myc* upstream region. The CAT activity in percentage acetylation per hr was assayed: pSP65, 1.0, 1.4; pDSS, 1.0, 1.5; p5-1, 1.0, 1.9; p5-2, 1.1, 2.1; p5-3, 1.2, 1.6; p5-4, 0.8, 1.7; and p5-5, 2.9, 4.3. (*B*) The competition assay was carried out as described in Fig. 3*B* except that p5-5 was used as the competing fragment instead of pNXS-1. The asterisk (*) next to p5-5 indicates that it was used as a competitor with both indicator genes. The CAT activity in percentage acetylation per hr was assayed: pSP65, 0.9, 1.3; and p5-5, 0.8, 1.1. H, *Hin*dIII; Sau, *Sau*3A I; Rs, *Rsa* I; Ac, *Acc* I; Ps, *Pst* I.

or subclone p5-5, there was no significant difference in its expression (Fig. 4*B*).

The experiments described above suggest the existence of trans-acting negative elements that down-regulate the expression of the c-*myc* gene. Early experiments involving the detection of DNase I-hypersensitive sites associated with highly active c-*myc* genes indicated that protein binding was required to achieve these high levels of expression (30). In the Burkitt lymphoma silent BL31, strong DNase I-hypersensitive sites are present near the promoters of the translocated c-*myc* allele, whereas they are absent in the silent germ-line allele. In order to test for the trans-acting factor(s) that might bind the sequences near the c-*myc* promoters (see Fig. 5), 3 μg of the c-*myc*/CAT indicator gene, pZHB2, were cotransfected with (*i*) 60 μg of vector alone; (*ii*) 30 μg of the vector alone plus 30 μg of subclone pRR100 (containing fragment −400 to −293) or subclone pRN (containing fragment −293 to +210) (indicator gene to competitor ratio, 1:10 by mass); or (*iii*) 60 μg of subclones pRR100 or pRN (1:20 by mass) (Fig. 5*A*). Cotransfection with either pRR100 or pRN reduced the activity of the indicator gene and, thus, appeared to titrate away a factor(s) required for the expression of the c-*myc* gene in a concentration-dependent manner (Fig. 5*A*). Since both of these competing fragments contain the sequences near the promoters, it is possible that nonspecific transcriptional factors, such as SP2 (31) or RNA polymerase II, were among the elements titrated away. To test this, we cotransfected 3 μg of the SV40/CAT indicator gene, pA10BB, with 60 μg of the vector alone or with the subclones pRR100 or pRN (Fig. 5*B*; see the legend to Fig. 3*B* for a description of the enhancerless early region SV40 promoter driving the CAT gene in pA10BB). In a competition assay with subclone pRN, which contains the c-*myc* promoters, there was decreased expression of the SV40-driven indicator gene. The competition assay with subclone pRR100 containing the more distal c-*myc* fragment had no effect on the SV40-driven indicator gene. Since this segment competes effectively with the c-*myc*-driven indicator gene, this result suggests that the distal fragment in subclone pRR100 contains a sequence(s) that binds a c-*myc*-specific positive factor. Since the pRN subclone competes with the SV40-driven indicator gene, it appears to interact with a nonspecific factor. Also shown in Fig. 5*A* is the competition assay in which the c-*myc*/CAT indicator gene competed with subclones p5-5 and pNXS-1, containing fragments that appear to be binding repressor elements (see Figs. 3 and 4). These were assayed as a function of the ratio of the indicator gene to competitor fragment. As one would predict, the c-*myc*/CAT indicator gene activity increased in a concentration-dependent manner.

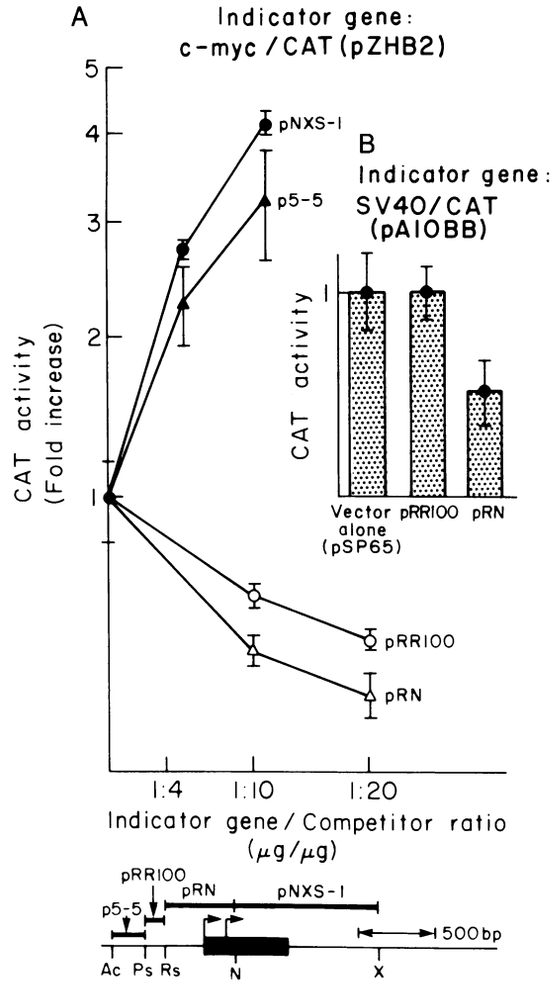

FIG. 5. The effect of the ratio between indicator gene and competing fragment on expression of the indicator gene. The competing fragments used in this experiment are indicated by the heavy lines above the partial map of the c-*myc* gene. (*A*) For the competition assay with p5-5 and pNXS-1, 5 μg of indicator gene pZHB2 was cotransfected with 50 μg of the pSP65 vector alone, 30 μg of the vector with 20 μg of either p5-5 or pNXS-1 (1:4 indicator gene/competitor ratio by mass), and 50 μg of either p5-5 or pNXS-1 (1:10 indicator gene/competitor ratio by mass). Again, the CAT activity is shown as fold increase over the activity measured with 50 μg of the vector alone, which is arbitrarily set at 1. The CAT activity in percentage acetylation per hr was assayed: pSP65, 0.3, 0.3; p5-5 (1:4), 0.5, 0.7; p5-5 (1:10), 0.7, 1.1; pNXS-1 (1:4), 0.7, 0.7; and pNXS-1 (1:10), 1.1, 1.2. For the competition assay with pRR100 and pRN, 3 μg of pZHB2 was cotransfected with 60 μg of the pSP65 vector alone, 30 μg of the vector with 30 μg of either pRR100 or pRN (1:10 indicator gene/competitor ratio by mass), and 60 μg of either pRR100 or pRN (1:20 indicator gene/competitor ratio by mass). The CAT activity in percentage acetylation per hr was assayed: pSP65, 0.76, 0.85; pRR100 (1:10), 0.41, 0.43; pRR100 (1:20), 0.28, 0.31; pRN (1:10), 0.23, 0.31; and pRN (1:20), 0.11, 0.20. (*B*) Three micrograms of indicator gene pA10BB (see the legend to Fig. 3) was cotransfected with 60 μg of the pSP65 vector alone, pRR100, or pRN. The CAT activity in percentage acetylation per hr was assayed: pSP65, 0.52, 0.83; pRR100, 0.62, 0.75; and pRN, 0.21, 0.40. Ac, *Acc* I; Ps, *Pst* I; Rs, *Rsa* I; N, *Nae* I; X, *Xba* I.

DISCUSSION

We have found sequences derived from the c-*myc* gene that induce or repress expression of a c-*myc*/CAT indicator gene in competition assays. The simplest explanation for these observations is that these sequences normally bind negative and positive regulatory factors that influence expression of the c-*myc* gene. Three regions, one approximately 400–600 bp upstream of the first promoter, a second in the first exon, and a third in the 5′ half of the first intron, contain sequences that compete for a putative negative regulatory factor(s). Recent studies of the murine c-*myc* gene by Remmers *et al.* (32), using a different experimental approach, identified a negatively acting sequence in a region roughly homologous to our first putative control region (see subclone P5-5, Fig. 4). The presence of these multiple intragenic sites that bind a putative negative regulatory factor(s) might explain why deletions of the upstream regions have very little effect on the expression of c-*myc*/CAT gene (Fig. 1). Furthermore, the negative control site we identify within the first intron might be responsible for the transcriptional elongation block observed by Bentley and Groudine (33) when HL60 cells are induced to differentiate. Its position fits well with a DNase-hypersensitive site that becomes apparent when these cells are induced to differentiate in the presence of retinoic acid. By contrast, we find a region approximately 290–400 bp upstream of the first promoter that competes for a putative positive factor(s). Further, a region located between posi-

tions −293 and +210 competes for a positive regulatory factor(s) but does not appear to be c-*myc* specific.

It has been proposed that the activation of the translocated c-*myc* gene in Burkitt lymphomas and murine plasmacytomas is due in part to the disruption of c-*myc* regulatory sequences (14). The binding sites of a negative regulatory factor(s) identified in this study are at or near the clustered breakpoints of translocations seen in most murine plasmacytomas and some Burkitt lymphomas. However, in a group of murine plasmacytomas in which the breakpoint is 350–500 bp upstream of the first exon (34), only the upstream binding site would be disrupted (assuming an analogous arrangement of control sequences in the mouse). Furthermore, translocations of c-*myc* have been characterized in which the breakpoint occurs either 3′ to the c-*myc* gene or 5′ to the upstream regulatory regions we have identified. Even in these distant translocations, it is possible that putative regulatory regions closer to the c-*myc* gene have been altered (35), but such findings suggest that additional factors play a role in deregulating the c-*myc* gene in these tumors.

We also cannot rule out the likely possibility that there are additional regulatory regions in the c-*myc* gene that we have failed to detect. Because we are using transient transfection, we are able to detect only those regulatory sequences for which the trans-acting factors are present at sufficiently high concentrations so that their effect will be evident even at the high copy number of the transfected genes. At the same time, however, the concentration of the trans-acting factor cannot be too high because under such circumstances competition will not occur. These trans-acting factors must also be able to bind to sequences in a supercoiled plasmid and, therefore, not in their normal chromosomal context. Finally, since our study was carried out largely in the Burkitt lymphoma cell line Ly65, we cannot be certain that other tumor cells would utilize identical regulatory sequences.

We have focused on the role of the c-*myc* regulatory sequences in the activation of the translocated allele. However, it is likely that sequences in the immunoglobulin loci to which the c-*myc* gene is translocated also play a role in c-*myc* activation. Cell hybrid studies have shown that the translocated c-*myc* gene is activated only in the B-cell background (36). In rare instances of Burkitt lymphoma (37) and murine plasmacytoma (38), the c-*myc* gene translocates to the immunoglobulin Ig heavy chain enhancer sequence. The fact that the immunoglobulin enhancer is usually not available to activate the translocated c-*myc* gene raises the possibility that there are other enhancer-like sequences or specific chromatin configurations in the immunoglobulin loci capable of activating the translocated c-*myc* gene (39).

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