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An Amino-Terminal c-Myc Domain Required for Neoplastic Transformation Activates Transcription

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The product of the c-*myc* proto-oncogene is a nuclear phosphoprotein whose normal cellular function has not yet been defined. c-Myc has a number of biochemical properties, however, that suggest that it may function as a potential regulator of gene transcription. Specifically, it is a nuclear DNA-binding protein with a short half-life, a high proline content, segments that are rich in glutamine and acidic residues, and a carboxyl-terminal oligomerization domain containing the leucine zipper and helix-loop-helix motifs that serve as oligomerization domains in known regulators of transcription, such as C/EBP, Jun, Fos, GCN4, MyoD, E12, and E47. In an effort to establish that c-Myc might regulate transcription in vivo, we sought to determine whether regions of the c-Myc protein could activate transcription in an in vitro system. We report here that fusion proteins in which segments of human c-Myc are linked to the DNA-binding domain of the yeast transcriptional activator GAL4 can activate transcription from a reporter gene linked to GAL4-binding sites. Three independent activation regions are located between amino acids 1 and 143, a region that has been shown to be required for neoplastic transformation of primary rat embryo cells in cooperation with a mutated *ras* gene. These results demonstrate that domains of the c-Myc protein can function to regulate transcription in a model system and suggest that alterations of Myc transcriptional regulatory function may lead to neoplastic transformation.

Unlike the functions of a number of other nuclear proto-oncogene products such as c-Fos (16, 17, 23, 28, 35, 48), c-Jun (5, 6, 29, 35, 44), and c-Myb (4, 24, 27, 50), the biochemical function of c-Myc remains unclear (8, 13). In fact, a number of studies have suggested that c-Myc might regulate transcription (21, 30, 32, 40), whereas others suggest a role for c-Myc in DNA replication (1, 18). In addition, c-Myc, a 439- or 454-amino-acid nuclear protein, shares sequence homology with a region of the adenovirus E1A protein and the simian virus 40 large T antigen that is necessary for the interaction of both of these viral proteins with the product of the retinoblastoma locus, Rb (14). The last observation adds to the broad range of possibilities for the mechanisms of action of c-Myc in normal and neoplastic cell growth.

The mechanism by which c-Myc contributes to cellular transformation is not clear. Through the study of c-*myc* mutants, it has been determined that regions from both the amino-terminal and carboxyl-terminal portions of c-Myc are required for transformation of rat embryo cells in cooperation with a mutated *ras* gene (39, 43). The carboxyl-terminal region of c-Myc contains a nonspecific DNA-binding domain (12), a nuclear targeting signal (9), and a potential specific DNA-binding domain containing a basic (b) region preceding both helix-loop-helix (HLH) and leucine zipper (ZIP) motifs (11, 27, 28, 33). To date only one transcription factor, TFE3 (3), has been found to contain the b-HLH-ZIP motif, although several other specific DNA-binding proteins contain either the b-ZIP or the b-HLH motif (20, 27). The amino-terminal c-Myc transforming region contains sequences which are conserved among the Myc family of proteins (43, 45); however, the function of this region is unknown. Because the amino-terminal c-Myc transforming region is rich in acidic, proline, and glutamine residues, we hypothesized that this region might be able to activate transcription when bound to DNA.

A DNA-bound LexA-Myc hybrid protein has been previously shown to activate transcription in yeast, although the transcriptional activation domain was not localized (25). Because no specific DNA-binding site is known for native c-Myc, and on the basis of the observation that activation domains of transcriptional factors can be localized by using chimeric molecules (22, 26, 50), we constructed GAL4/c-Myc chimeras to identify potential c-Myc activation domains. We report here the identification of a domain of human c-Myc known to be necessary for neoplastic transformation that can activate transcription effectively in mammalian cells when bound to DNA via the DNA-binding domain of the yeast transcriptional activator GAL4 (26, 36). This observation is consistent with the idea that c-Myc is a transcription factor containing separable transcriptional activation and putative specific DNA-binding domains (20, 27, 34). Moreover, these results suggest a correlation between transcriptional activation and c-Myc transforming activity.

MATERIALS AND METHODS

Plasmid constructions. The GAL4(1–147) activator vector and the G5E1bCAT reporter plasmid have been described elsewhere (26). The multiple cloning site of GAL4(1–147) (a gift of I. Sadowski) was replaced with synthetic oligonucleotides (creating the GALO and GALM plasmids) with appropriate sites to permit c-*myc* subcloning in frame at the 3′ end of the *GAL4* gene fragment. Human cDNA c-*myc* gene fragments were excised from pR*myc* (a gift from A. Shatzman, R. Watt, and M. Rosenberg) (46), pOTS*myc* (12, 46), or pBS0/1-Mut15 (a gift of R. Eisenman) plasmids and were inserted into the modified *GAL4* plasmids at the new multiple cloning sites. Restriction of pR*myc* or pOTS*myc* with *Nde*I yielded DNA fragments with 5′ ends corresponding to

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codon 1 of c-*myc* (because of the linker DNA sequences, pOTS*myc* yields four extra amino acids at the c-Myc amino terminus, but pR*myc* yields no additional amino acids at the amino terminus). Restriction of pBS0/1-Mut15 with *Nco*I yields 15 additional amino acids of c-Myc initiated at the upstream alternative CTG translational start codon (18). Internal c-*myc* restriction sites utilized were *Pst*I, *Bst*EII, *Cla*I, and *Xho*I sites that were created by linker insertion as described previously (43).

The putative specific c-Myc DNA-binding site 5′ of the c-*myc* gene reported by Iguchi-Ariga and co-workers (19) was subcloned into chloramphenicol acetyltransferase (CAT) plasmids to test for the ability of c-Myc to transactivate. The 200-bp *Hin*dIII-*Pst*I fragment approximately 2 kb upstream of c-*myc* exon 1 was ligated into *Hin*dIII and *Pst*I sites 5′ of the adenovirus E1b promoter in the reporter E1bCAT (26), creating HPM-E1bCAT, and 5′ of the GAL4-binding site in the reporter G1E1bCAT, creating HPM-G1E1bCAT (26).

Cell culture and transfection. Two micrograms of activator plasmid, 2 μg of reporter plasmid, and 2 μg of pCH110 (a eucaryotic β-galactosidase-constitutive expression vector used to normalize for transfection efficiency; Pharmacia) were cotransfected into 100-mm-diameter plates of 50% confluent CHO-DUKXBII cells by the DEAE-dextran technique, and the cells were harvested 48 h after dimethyl sulfoxide shock and assayed for CAT activity as described elsewhere (26, 38, 41). Relative activities were measured by phase extraction assay using butyryl coenzyme A (41), normalized to β-galactosidase activity (38), and adjusted to designate the strongest activator as 100. Quantities of cell extract used for the thin-layer chromatography (TLC) assay were adjusted for β-galactosidase activity. For the experiment whose results are shown in Fig. 1C, relative activities were measured by scraping the spots of acetylated chloramphenicol from the TLC plate, counting them in a liquid scintillation counter, and normalizing the activity of the strongest activator to 100. Otherwise, quantification of relative CAT activities was determined by the phase extraction assay.

CHO cells stably transfected with G1E1bCAT reporter plasmid were generated by transfecting CHO cells (50% confluent 100-mm-diameter plates) with 1 μg of pSV2neo, which confers neomycin resistance (42), and 20 μg of G1E1bCAT with Lipofectin (Bethesda Research Laboratories, Gaithersburg, Md.) according to the instructions of the manufacturer. Cells were grown in α minimal essential medium containing 10% fetal calf serum and 0.2 mg of (active) G418 per ml of medium for 48 h after transfection. Three weeks after initiation of G418 selection, approximately 120 foci of G418-resistant cells were pooled for analysis to avoid confounding effects of specific chromosomal integration. For the activation assay, the pooled cell lines were grown in the presence of G418 to 50% confluency. The 100-mm plates were transfected with 2 μg of activator plasmids as described above with DEAE-dextran. CAT assays were performed as described above.

COS7 cells transfected by the DEAE-dextran procedure were labeled 48 h after transfection with 110 μCi of [^{35}S]methionine ml^{-1} for 4 h. Subsequently, immunoprecipitation was performed with Pansorbin (protein A-bound *Staphylococcus aureus*; Calbiochem-Behring) and rabbit anti-GAL4 antiserum (a gift of I. Sadowski), and polyacrylamide gel electrophoresis was performed as described previously (26). Immunofluorescent microscopy of transfected COS7 cells was performed as described previously (10).

RESULTS

Identification of a c-Myc activation region. To study possible transcriptional activation characteristics of c-Myc, we used the eucaryotic expression plasmid vector GAL4(1–147), containing the simian virus 40 early promoter, a gene fragment that encodes the DNA-binding domain of GAL4, and the simian virus 40 polyadenylation site (36). The multiple cloning site between the GAL4(1–147) gene fragment and the polyadenylation site was modified with synthetic oligonucleotides containing sites compatible with c-*myc* DNA restriction endonuclease fragments. The c-*myc* DNA fragments were then spliced into the new multiple cloning site, resulting in *GAL4*/c-*myc* fusion genes. These activator plasmids were transiently transfected into Chinese hamster ovary cells along with the reporter plasmid G5E1bCAT, which contains five copies of the GAL4-binding site upstream from the adenovirus E1b promoter driving the CAT gene (Fig. 1A) (26). This E1b region is a minimal promoter containing a simple TATA sequence (26).

As an internal control for transfection efficiency, cotransfections also included pCH110, a plasmid that constitutively expresses a bacterial β-galactosidase gene behind a simian virus 40 early promoter. The β-galactosidase activity of the cell lysates was measured by a colorimetric assay. CAT enzyme activity was assayed by TLC and by phase extraction assay, and CAT activity was normalized to β-galactosidase activity. Various c-*myc* portions were fused to the GAL4(1–147) carboxyl terminus, with two constructs also containing additional internal deletions. The chimeric protein designations consist of GM followed by the included Myc amino acids in parentheses. Internally deleted amino acid designations consist of D followed by the removed amino acids. The relative transcription activation strengths of representative GAL4/c-Myc chimeras were determined by the TLC CAT assay (Fig. 1B). Efficient transactivation by the activator constructs required the cognate GAL4 DNA-binding sequence (Fig. 1C).

Fusions of amino terminal segments, but not carboxyl-terminal segments, of c-Myc to GAL4(1–147) activate transcription of the reporter gene (Fig. 2). We found that the fusion of the GAL4 DNA-binding domain to the c-Myc fragment lacking amino acids 373 to 439 (the leucine zipper oligomerization domain), named GM(1–372), yielded measurable transcriptional activation of the CAT gene (Fig. 2). GM(1–262) is a chimera which encodes the first 262 Myc amino acids, which lack the nuclear targeting signal, the nonspecific DNA-binding domain, and the b-HLH-ZIP domain. This construct is a stronger activator than GM(1–372). Deletion of residues 106 to 143 from residues 1 to 262 in GM(1–262)D106–143 did not appear to affect transactivation, whereas removal of amino acids 41 to 178 in GM(1–262)D41–178 drastically reduced transcription activation (Fig. 2). GM(1–143) also showed activation properties. Constructs which failed to activate include GM(143–262), which contains a highly acidic region of c-Myc, and GM(262–439), which contains the carboxyl b-HLH-ZIP motif (Fig. 1B and 2). These data define a transactivation domain contained between amino acids 1 and 143 of c-Myc.

Sublocalization of c-Myc activation regions and their interactions. The amino-terminal activation domain of c-Myc was further subdivided arbitrarily into three independent regions by restriction sites in this coding sequence. Each region (A [amino acids to 41], B [amino acids 41 to 103], and C [amino acids 103 to 143]) was capable of activating transcription. Region A, which contains the glutamine-rich, slightly acidic

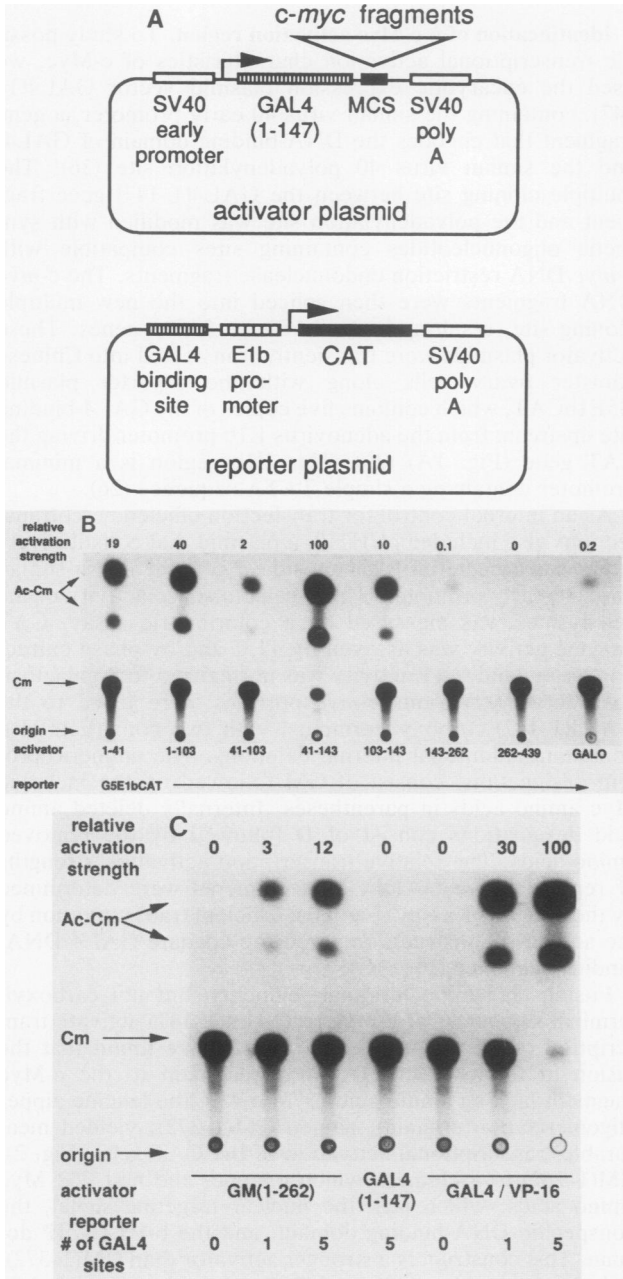

FIG. 1. Transcriptional activation by DNA-bound c-Myc. Relative CAT activity was quantitated and is indicated above each track of the autoradiograms. (A) Diagram of GAL4/c-Myc activator and reporter plasmids. MCS, Multiple cloning site; SV40, simian virus 40. Figure not drawn to scale. (B) Relative transactivation strengths of selected GAL4/c-Myc hybrid activators. A reporter CAT plasmid containing five GAL4 sites, G5E1bCAT, was cotransfected with various plasmids encoding hybrid activators. Note that GM(143–262) weakly activates CAT activity, but less than the background of its vector, GALO. (C) GAL4 sites increase activation by GAL4-Myc plasmid GM(1–262). The activator plasmid GM(1–262) was cotransfected with reporter plasmids bearing zero, one, or five GAL4 DNA-binding sites. Comparisons are made with the GAL4(1–147) negative control and the GAL4-VP16 positive control. Cm indicates ^{14}C-labeled chloramphenicol, and Ac-Cm indicates the acetylated forms of chloramphenicol.

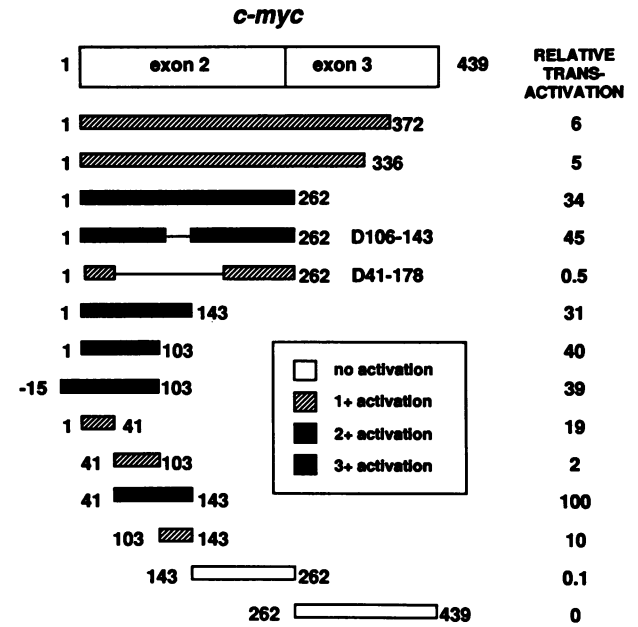

FIG. 2. Relative transactivation strengths of various GAL4/c-Myc chimeras. The bars in the diagram depict the different fragments of c-Myc that were fused to the carboxyl terminus of the amino-terminal 147 amino acids of GAL4. The amino acid numbers beginning from the ATG start codon are indicated. The chimera starting at −15 corresponds to the c-Myc polypeptide from an alternative CTG translational start site. The interrupted bars indicate internal deletions in the fragment containing amino acids 1 to 262. Shading indicates relative transactivation strength as indicated in the inset: 0.5 to 25% of the maximal strength is designated 1+, 26 to 50% is designated 2+, and 51 to 100% is designated 3+. The quantitative relative transactivation strength obtained by a phase extraction CAT assay performed as described elsewhere (41) is indicated at the right. CAT activity was normalized to β-galactosidase activity, with results taken from the average of two experiments, and then adjusted to designate the peak transactivation activity of GM(41–143) as 100.

41 amino acids, and region C both activated transcription in GAL4 fusion proteins (Fig. 1B and 2). Region B, which contains the proline-rich segment, transactivated very weakly, but the linkage of this proline-rich element augmented transactivation by either of the last two elements [creating GM(1–103) and GM(41–143)] (Fig. 1 and 2). In fact, peak activation was provided by a fusion protein containing regions B and C, GM(41–143). Activation by regions A and B or B and C appeared to be higher than expected when they were joined covalently in GM(1–103) or GM(41–143) (Fig. 2).

Utilization of an alternative translational start codon does not alter activation properties. In vivo c-Myc protein synthesis also initiates at a CTG site located 15 codons upstream of and in frame with the conventional ATG start site (18). To test whether this alternative start site would yield different transcriptional activation behavior, a mutant construct of c-*myc* (pBS0/1-Mut15) in which this CTG was altered to ATG was obtained, yielding a new *Nco*I site (a gift from R. Eisenman). A construct containing these 15 codons plus the first 103 codons of c-*myc* was compared with a similar construct without the additional 15 codons. No difference was seen in the level of activation of CAT expression (Fig. 2).

Potency of the c-Myc activation domain. The GAL4/c-Myc chimera GM(41–143) is a potent activator. GAL4/VP16, a

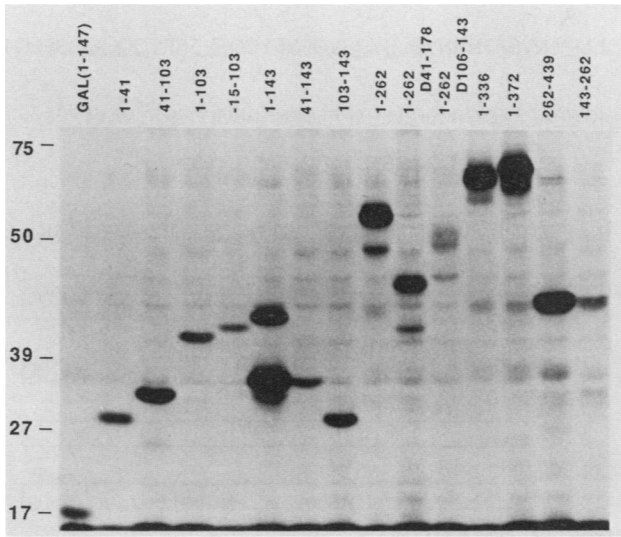

FIG. 3. Protein levels of GAL4/c-Myc chimeras. [^{35}S]methionine-labeled GAL4/c-Myc derivatives from transfected COS7 cells were immunoprecipitated with an anti-GAL4(1–147) antibody (a gift of I. Sadowski) and separated on a sodium dodecyl sulfate–10% polyacrylamide gel (26). The transfected activator [numbers above the gel correspond to the c-*myc* codons attached to GAL4(1–147), and D indicates the internally deleted c-*myc* codons] is indicated above each lane of the autoradiogram. The positions of molecular mass markers are indicated on the left in kilodaltons. Note that GM(1–143) also yielded a smaller, presumably proteolytic 32-kDa polypeptide.

fusion protein which is the strongest known chimeric activator, is only two- to threefold more active than GM(41-143). In this case, the fusion protein contains the GAL4 DNA-binding domain linked to the acidic activation domain of VP16 from herpes simplex virus (a gift from I. Sadowski) (36). We estimate GM(41–143) to be 15- to 20-fold more active than the native GAL4 activating region (26) (data not shown). The high potency of the c-Myc activating region argues that its activity is unlikely to be an artifactual result of fusion to GAL4(1–147). In addition, the c-Myc activating region does not derive its potency in this assay solely on the basis of an acidic charge.

Lack of activation by certain GAL4/c-Myc chimeras is not due to reduced activator protein levels. Relative amounts of the fusion proteins were measured in order to determine whether protein levels are responsible for the differences in transcriptional activation by the various constructs. Because the GAL4(1–147) vector provides a low yield of fusion proteins in CHO cells (26), COS7 cells were chosen for transient transfection and metabolic labeling with [^{35}S]methionine. Immunoprecipitations of the lysates were performed with anti-GAL4 antiserum (Fig. 3). This confirms the presence of immunoreactive proteins with the predicted relative electrophoretic mobilities for each GAL4/c-Myc chimeric protein. Most of the chimeric genes yielded levels of proteins comparable to that of the unfused GAL4(1–147) (Fig. 3). Several of the constructs appeared to produce high levels of the chimeras [GM(1–143), GM(1–262), GM(1–262)D41–178, GM(1–336), GM(1–372), and GM(262–439)]; however, these constructs did not yield the highest transactivation activity. Similar relative abundance of the chimeric proteins was detected by immunoblotting of transfected CHO cell extracts with anti-GAL4 antibody, although a high background level was observed (data not shown). This demonstrates that the lack of transactivation in the constructs that did not transactivate was not due to the absence of the hybrid GAL4/c-Myc proteins. Moreover, indirect immunofluorescent microscopy of COS7 cells transfected and subsequently stained with anti-GAL4 antibody revealed that the transcriptionally inactive GAL4/c-Myc chimeras, GM(143–262), GM(262–439), and GM(1–262)D41–178, localized properly to the nucleus as efficiently as the active GAL4/c-Myc chimeras (data not shown).

c-Myc nonspecific DNA-binding domain inhibits activation of extrachromosomal reporter genes. It is noteworthy that certain constructs had unexpectedly low transactivation activity in our model system. Specifically, GM(1–372) and GM(1–336), which both retain the nonspecific DNA-binding domain of c-Myc (12), were less active than GM(1–262), which lacks this domain (Fig. 2). The relative decrease in the activities of chimeras containing the c-Myc nonspecific DNA-binding domain might be the result of (i) differences in protein levels, (ii) protein conformational differences, or (iii) the binding of the c-Myc nonspecific DNA-binding domain to nonspecific chromosomal DNA-binding sites which compete with specific binding to the GAL4 sites on the reporter plasmid. It is noteworthy that protein levels of GM(1–262), GM(1–372), and GM(1–336) are comparable (Fig. 3).

We hypothesized, on the basis of previous studies of facilitated diffusion in procaryotes (49), that the nonspecific DNA-binding activity of GM(1–336) or GM(1–372) could serve to contact DNA initially, allowing the protein to scan DNA to locate its specific binding site. In such case, a large DNA molecule might function to funnel the activators to a specific binding site contained in the molecule. Conversely, chromosomal DNA without specific sites could compete for nonspecific binding by drawing activators away from small extrachromosomal plasmids containing the binding site. If the decrease in activation potential by GM(1–336) or GM(1–372) relative to GM(1–262) occurs because of competition by chromosomal DNA with extrachromosomal specific DNA-binding sites via nonspecific DNA binding, then these differences should abate if chromosomally integrated copies of the reporter are employed for the activation assay. If the differences are due to inherent conformational changes in the chimeric proteins, then the relative activities of the chimeras should not depend on the state of the reporter gene.

Indeed, when pooled stably transfected CHO cells carrying integrated G1E1bCAT as a reporter were used, GM(1–262) was less active than GM(1–336) (Fig. 4). In contrast, GM(1–262) was much more active than GM(1–336) in the transient transfection assay using G1E1bCAT (data not shown) or G5E1bCAT (Fig. 2). This result suggests that competition by chromosomal DNA for nonspecific DNA binding by GM(1–372) and GM(1–336) may result in reduced binding to the extrachromosomal reporter plasmid in this transient transfection assay system. Although it is possible that the GAL4-binding domain interacts differently with binding sites present as extrachromosomal copies as opposed to stably integrated copies, these results are consistent with the hypothesis that the nonspecific DNA-binding domain may serve physiologically to enhance the rate at which c-Myc locates its target chromosomal DNA sequence through a mechanism of facilitated diffusion to activate transcription (49).

c-Myc does not activate CAT through a putative specific DNA-binding site in CHO cells. In an attempt to delineate the significance of the c-Myc activation domain in native c-Myc, we sought to determine whether c-Myc expression vectors can activate the CAT gene through a putative c-Myc specific

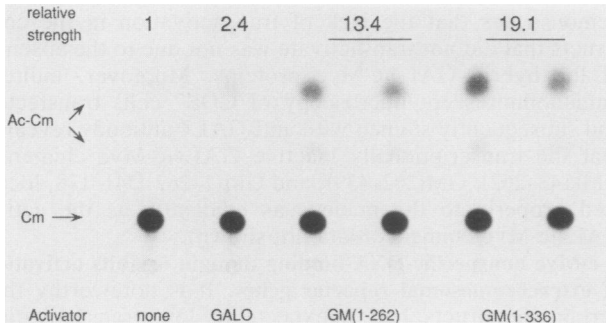

FIG. 4. Activation of chromosomally integrated reporter G1E1bCAT by GM(1–262) and GM(1–336), which contain the c-Myc nonspecific DNA-binding domain. Comparison is made with the absence of an activator and with GALO, which produces the DNA-binding domain GAL4(1–147). Relative CAT activities were quantitated by phase extraction assays for duplicate experiments and are given above the autoradiogram of the TLC CAT assay. Cm, [^{14}C]chloramphenicol; Ac-Cm, acetylated [^{14}C]chloramphenicol.

DNA-binding site reported by Ariga and co-workers (1, 19). We placed a 200-bp genomic fragment containing the putative c-Myc specific DNA-binding site 5′ of the E1b promoter and CAT gene in two different constructs. HPM-G1E1bCAT contains a GAL4-binding site 3′ of the putative Myc binding site, whereas HPM-E1bCAT lacks the GAL4-binding site. We failed to detect any baseline activation of the reporters HPM-E1bCAT and HPM-G1E1bCAT by endogeneous CHO cell c-Myc proteins. In addition, c-Myc expression vectors Rous sarcoma virus-human c-*myc* cDNA (in which c-*myc* cDNA is driven by the Rous sarcoma virus long terminal repeat [15]) or Moloney murine leukemia virus human c-*myc* (which contains all three exons of c-*myc* driven by the Moloney murine leukemia virus long terminal repeat [43]) failed to activate the CAT gene in both HPM-E1bCAT and HPM-G1E1bCAT in cotransfection experiments. In contrast, the CAT gene in HPM-G1E1bCAT can be activated by GAL4-VP16 or GM(1–262) through the GAL4-binding site (data not shown).

DISCUSSION

Our results demonstrate that domains of c-Myc protein that are necessary for transformation can activate transcription in a model system. Similar conclusions have been derived from observations on Myb and Rel domains required for oncogenic transformation and gene activation (22, 24). Large deletions within two c-Myc regions, amino acids 1 to 143 and 321 to 439, abolish or dramatically reduce its ability to cooperate with EJ-Ras to transform primary rat embryo cells (17, 43). While the carboxyl-terminal domain necessary for Myc to cooperate in the transformation of rat embryo cells corresponds to the b-HLH-ZIP region, the amino-terminal region necessary for cotransformation exactly coincides with the domain which we have demonstrated to have transactivation properties (Fig. 5B). This suggests a correlation between transcriptional activation and c-Myc transforming activity. Our identification of an amino-terminal c-Myc activation domain indicates that this c-Myc region has the potential to interact with the transcriptional machinery in the context of the native c-Myc protein. Whether this potential interaction(s) results in gene activation, suppression, as with c-*myc* autoregulation (31), or both effects remains to be determined.

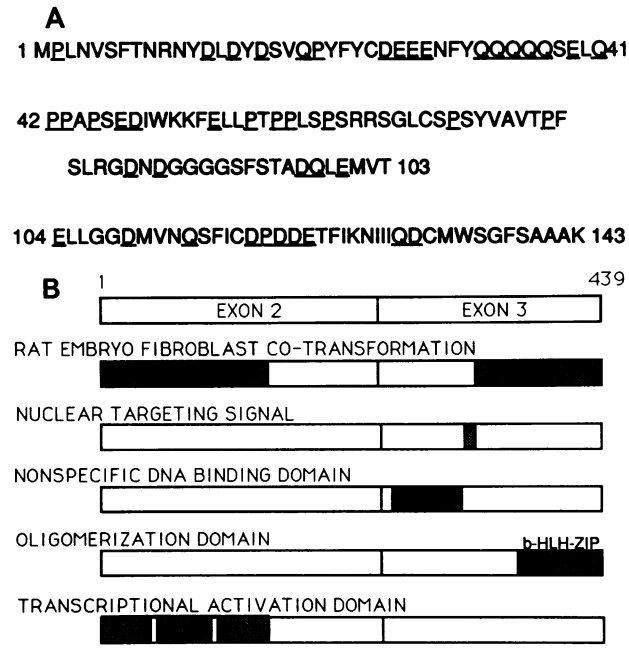

FIG. 5. (A) Amino acid sequence of the c-Myc transcriptional activation domain (2). Prolines, glutamines, and acidic residues are underlined. (B) Functional anatomy of the c-Myc protein. The top bar depicts c-Myc amino acids 1 to 439 encoded by exons 2 and 3. The next five bars show regions of c-Myc required for rat embryo cell cotransformation, nuclear localization, nonspecific DNA binding, oligomerization, and transcription activation.

Like some other transactivators (20, 27, 47), c-Myc appears to possess a transactivation domain that can arbitrarily be subdivided by restriction endonuclease sites into independent regions. The first region, A, contained in amino acids 1 to 41, includes a 22-amino-acid segment with a 32% glutamine content and a small acidic region (Fig. 5A). The second region, B, contained in amino acids 41 to 103, includes a highly conserved 21-amino-acid region containing 33% proline. This segment is nearly identical in c-Myc, N-Myc, L-Myc, and s-Myc (45). The third region, C, contained in amino acids 103 to 143, also has a stretch of amino acids with perfect homology to the other members of the Myc family. However, this segment bears no resemblance to any previously described transactivation domain motif.

The c-Myc protein contains a highly acidic region between amino acids 242 and 263 reminiscent of the acidic activation domains of several well-characterized transcriptional activators (34). Although we anticipated that this fragment might activate transcription, no significant activation was observed with GM(143–262) in the context of the GAL4 chimera in CHO cells. Since some degree of cellular specificity has been demonstrated in other transactivation assays (47), it is possible that the c-Myc acidic region activates transcription in a different model system. In fact, utilization of a more complex promoter other than the minimal E1b promoter from adenovirus used in our model system might identify some additional regions of c-Myc which may influence transcriptional activation. However, it is notable that the c-Myc acidic region is not required for rat embryo cell cotransforming activity of c-Myc (43).

The c-Myc nonspecific DNA-binding domain (Fig. 5B), which may enhance transcriptional activation by native c-Myc protein, is not required for oncogenic transformation

of rat embryo cells (9, 43), but it is required for the inhibition of differentiation of fibroblast 3T3-L1 cells into adipocytes (15). These functional differences may depend on the level of c-Myc proteins expressed in these assays. In the case of rat embryo cells c-Myc is highly expressed (43), whereas in Swiss 3T3-L1 expression of the exogenous c-*myc* gene is difficult to detect (15). Curiously, the c-Myc nonspecific DNA-binding domain contains four repeats of the SPXX motif that is required for histone H1 to bind to the minor groove of AT-rich DNA regions (7). Our preliminary studies of the nonspecific DNA-binding domain suggest that a differential effect of this domain can be observed in transient reporter assays when compared with stably transfected reporter cells. This effect may be due to the ability of this domain to bind DNA nonspecifically, and in this way the domain may enhance the targeting of c-Myc to a specific binding site by facilitated diffusion (49). Similar protein regions that modulate transcription activation have been observed in c-Jun (5) and c-Myb (37). However, regions of c-Jun and c-Myb that might interact with DNA nonspecifically have not been determined.

The functional domains of c-Myc identified to date (Fig. 5B) suggest the following model of c-Myc activity in vivo. c-Myc is synthesized in the cytoplasm and undergoes oligomerization (11), either homo-oligomerization or hetero-oligomerization, with another protein. Its nuclear targeting sequence provides a signal for its transport into the nuclear compartment (9). At low physiological levels, c-Myc binds DNA nonspecifically and "slides" and "jumps" (49) to enhance its rate of location of its specific DNA-binding sequence in the vicinity of its target gene or genes, which are recognized by the b-HLH-ZIP motif. It then activates or inhibits transcription of its target genes, with consequent alterations in cell proliferation. Although a specific DNA sequence to which c-Myc binds was recently reported (1), we have not been able to confirm these observations by using an identical binding site. Identification of a c-Myc DNA-binding sequence and its associated genes will provide the opportunity to directly validate this model.

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