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DNA Binding and Transactivation Activity of A-*myb*, a c-*myb*-related Gene[1]

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ABSTRACT

A partial-length A-*myb* complementary DNA recently cloned by low-stringency hybridization with a c-*myb* probe to complementary DNA libraries derived from human cell lines showed a high degree of homology with the DNA-binding domain of c-*myb* and B-*myb*, suggesting that A-*myb* also encoded a DNA-binding protein. We report here the sequence of the entire coding region of A-*myb* complementary DNA and show that the full-length GST-A-myb fusion protein or a truncated derivative corresponding only to the putative DNA-binding domain interacts specifically with Myb-binding sites of the c-*myb* responsive promoters, MIM-1 and CD34. In transient transfection assays, A-*myb* transactivated the bound promoters. These results suggest that, analogous to the other members of the Myb family, the A-*myb* gene encodes a bona fide transactivator. The distinct function of A-*myb* might derive from its pattern of expression and/or its relative potency as a transactivator of *myb* target genes.

INTRODUCTION

Two c-myb-related genes, A-*myb* and B-*myb*, have recently been identified which are homologous to c-*myb* in the DNA-binding domain and are abundantly expressed in many cell types including hematopoietic cells, epithelial cells, and fibroblasts (1). Like c-*myb*, B-*myb* appears to be involved in transcriptional regulation by direct interaction with Myb-binding sites (2). B-*myb* might also be a c-*myb* functional equivalent in nonhematopoietic cells since introduction of a constitutively expressed B-*myb* cDNA[3] into BALB/c3T3 mouse fibroblasts results in reduced growth factor requirements and activation of cdc2 and cyclin D1 expression (3). The biochemical properties of A-*myb* are unknown, and there are no functional studies that support a role similar to that of c-*myb;* on the contrary, the reported decline of A-*myb* mRNA levels upon exit from G_0 in normal T lymphocytes raises the possibility that A-*myb* negatively regulates cell proliferation (4). To address these possibilities, we cloned the full-length A-*myb* cDNA and analyzed its DNA-binding and -transactivating properties. Our results suggest that A-*myb* binds to and transactivates promoters containing Myb-binding sites, properties compatible with a c-*myb*-like function in cells that do not express c-*myb*. In cells that coexpress A-*myb* and c-*myb*, A-*myb* could either potentiate or weaken c-*myb* activity depending on the relative potency of each gene as a positive regulator of Myb-responsive genes.

MATERIALS AND METHODS

Cloning of a Full-Length A-*myb* cDNA. The 5′ region of A-*myb* cDNA from nt 1 to 1300 cloned in the pUC18 plasmid (pUCA-myb *Nco*I) was a kind gift from Dr. N. Nomura. The segment of A-*myb* cDNA extending from nt 1300 to 2341 was amplified by reverse transcriptase-PCR from RNA of the CCRF-CEM T-leukemia line and subcloned into the TA-cloning plasmid p2000. The PCR fragment was sequenced, shown to be identical to the published sequence, ligated to the pUCA-myb *Nco*I plasmid using *Nco*I and *Sca*I sites, and designated pUCA-myb *Sca*I. This partial-length A-*myb* cDNA was then cloned into the pSV40 polylinker vector and named pSVA-myb *Sca*I. To reconstitute the remaining 3′ segment of the A-*myb* cDNA, a genomic library from peripheral blood lymphocytes was screened with the most 3′ fragment of the A-*myb* cDNA. Three positive clones were isolated and found to be identical by restriction enzyme analysis. A 1.3-kilobase *Hind*III fragment hybridized with an 18-base oligomer corresponding to the most 3′ region of the partial-length A-*myb* cDNA. Sequence analysis of this 1.3-kilobase *Hind*III fragment revealed a 109-nt sequence overlapping with the published 3′ A-*myb* cDNA sequence, followed by 22 nt of new A-*myb* coding sequence and a TAA stop codon. An 800-base pair *Eco*RV-*Hind*II segment included in the 1.3-kilobase *Hind*III genomic subclone was then inserted into pSV-A-myb *Sca*I digested with *Eco*RV and *Sma*I, generating the full-length pSV-A-myb cDNA.

Cloning of HA-tagged A-*myb* Full-Length cDNA. The full-length A-*myb* cDNA was tagged by insertion of a triple tandem 9-amino acid-HA epitope (YPYDFVPDYA) (5, 6). The TGA stop codon upstream of the translation initiation ATG codon of A-*myb* was eliminated by PCR amplification using a mutated 5′ oligomer and a 3′ oligomer corresponding to nt 639–665. The PCR product was cloned into the PCR 2000 vector (In Vitrogen, San Diego, CA), sequenced, and cloned back into SKA-myb using *Xho*I and *Nde*I restriction sites. This plasmid is designated SKA-myb 4. SKA-myb HA was constructed by inserting a double-stranded synthetic oligonucleotide encoding a single epitope, preceded by the Kozak's consensus sequence for proper initiation (5′T CGAGGCCACCATGGCTTACCCATACGATGTTCCAGATTACGCT-TG 3′) (3′ CCGGTGGTACCGAATGGGTATGCTACAAGGTCTAATGCG-AACAGCT-5′) SKA-myb 5′ HA3 was made by inserting two additional in-frame copies of the epitope at the *Sal*I restriction site (6).

Cloning of A-*myb*/c-*myb* Chimeric Constructs. The chimeric SKA-myb-DBc-*myb* construct containing the A-*myb* DNA-binding domain (nt 1–755) linked to the c-*myb* transactivating domain (nt 1201–2450) was cloned as follows: (*a*) the c-*myb* fragment that includes the transactivating domain was obtained from pSV-c-myb (10) by *Nde*I restriction digestion, blunt-ending, and a second digestion with *Hind*III; the fragment was ligated into the Bluescript vector at the *Hind*II and *Hind*III sites, and the plasmid was called SKTADc-myb; (*b*) pSKTADc-myb was digested with *Hind*III, Klenow-filled with dGTP, dATP, and dCTP only, and redigested with *Xba*I; and (*c*). The A-myb fragment containing the DNA-binding domain was obtained from pUC18Amyb *Sca*I by *Nde*I digestion, Klenow filling with dTTP, digestion with *Xba*I, and ligation of the resulting fragment into pSKTADc-myb.

The chimeric SKc-mybDBA-myb construct containing the c-*myb* DNA-binding domain (nt 1–1200) followed by the A-myb putative transactivation domain (nt 754–2463) was constructed by digesting pSKAmyb with *Sal*I, blunt-ending with Klenow enzyme, redigesting with *Nde*I, followed by Mung bean nuclease digestion. The c-*myb* DNA-binding domain was obtained from pSVc-myb (10), digested with *Nco*I, *Hin*dIII, and blunted with the Klenow enzyme. This c-*myb* fragment was ligated into the SKAmyb vector. Sequence analysis confirmed that the constructs were in the correct reading frame. Both chimeric constructs were cloned into the pSV polylinker expression vector by blunt-end cloning at the *Eco*RV site.

Generation of Recombinant GST-A-*myb* Plasmids. The A-*myb* cDNA segment from nt90 to 760, which covers the putative DNA-binding domain and includes the *Bam*HI and *Eco*RV restriction sites, was amplified by PCR. This PCR product was purified, digested with *Bam*HI and *Eco*RV, ligated to the Bluescript SK vector, and designated SKAmyb3R. The A-*myb* DNA-binding domain was excised with *Bam*HI and *Eco*RV, ligated to pGEX-2TK linearized with *Eco*RI and blunt-ended with the Klenow fragment, and digested with *Bam*HI. This plasmid was named GST A-mybDB. To obtain the full-length GST-A-myb construct, the full-length A-*myb* cDNA was cloned into the SK vector (SKA-myb1), digested with *Cla*I and *Sma*I, and ligated to SKAmyb3R

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[3] The abbreviations used are: cDNA, complementary DNA; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate; PBS, phosphate-buffered saline; HA, hemagglutinin; nt, nucleotide; NETN, 20 mM Tris-HCl (pH 8.0)-100 mM NaCl-1 mM EDTA-0.5% Nonidet P-40; CAT, chloramphenicol-acetyl transferase; GST, glutathione *S*-transferase.

digested with *Cla*I and *Hin*dII. This plasmid was named SKAmyb3. SKAmyb3 was digested with *Xho*I, blunt-ended with Klenow enzyme, and redigested with *Bam*HI. This *Xho*I-*Bam*HI fragment was ligated to the pGEX-2TK vector digested with *Eco*RI, blunt-ended with the Klenow enzyme, and digested with *Bam*HI. The full-length GST-A-myb plasmid was named GST-A-myb2.

Expression and Purification of Glutathione *S*-Transferase Fusion Proteins. Glutathione *S*-transferase fusion protein expression and purification were essentially as described (7). Fresh overnight cultures of *Escherichia coli* (DH5a; Bethesda Research Laboratories) transformed with one of the above-mentioned plasmids were diluted 1:10 in Luria-Bertani medium containing ampicillin and incubated for 4 h at 37°C with shaking. After 2 h of culture, isopropyl-β-D-thiogalactopyranoside (Bethesda Research Laboratories) was added to a final concentration of 0.1 mM, and the incubation was continued for 3 h at room temperature. The induced bacterial cultures were collected by centrifugation at 4000 × *g* for 5 min at 4°C and resuspended in one-tenth of the original volume in NETN. Bacteria were then lysed in ice by mild sonication and centrifuged at 10,000 × *g* for 10 min at 4°C.

The resulting supernatant was rocked for 15–30 min at 4°C with 25 μl/ml of glutathione-Sepharose beads, prewashed three times, and resuspended (final concentration, 1:1 v/v) in NETN containing 0.5% powdered milk. The glutathione-Sepharose beads were then washed three times with NETN and the bound material was eluted by rocking the Sepharose for 20–30 min in 20 mM reduced glutathione and 50 mM Tris-HCl (pH 8.0) at 4°C on a rotating platform for 15 min. Supernatant containing the fusion protein was collected by brief centrifugation. For analysis of bound bacterial proteins, supernatants were boiled in 1× sample buffer [2% SDS-10% glycerol-62 mM Tris (pH 6.8)] and loaded onto SDS-polyacrylamide gels. Proteins were visualized by Coomassie blue staining and quantitated by Bradford protein microassay (Bio-Rad).

Gel Retardation Assay. Different amounts of GST-A-myb fusion protein were mixed with a ^{32}P-labeled double-stranded oligonucleotide including the Myb-binding site (Fig. 3, *Box A*) of the Myb-responsive MIM-1 promoter (8) or two Myb-binding sites (nt +67 to +102) of the human CD34 promoter (9), and gel retardation assays were performed as described (10). The *Box A* oligonucleotide of the MIM-1 promoter (5′ TCGACACATTATAACGGTTTTTTAGC 3′) was used as specific competitor. The underlined consensus sequence TAAC was mutated to TGGG and used as irrelevant competitor. The CD34 oligonucleotide from +67 to +102 (5′ TTTGGGACCAACCAGGGGAGCTCAAGTTAGTAGCAG 3′) was used as specific competitor. The underlined consensus sequences CAAC and GTTA were mutated to TGGC and GCCC, respectively. The mutated oligomer was used as an irrelevant competitor.

RNA Isolation and Northern Blot Analysis. Total cellular RNA was purified from transfected cells as described (11). RNA blotting to nylon filters and hybridization to a ^{32}P-labeled 1.0-kilobase A-*myb* insert (nt 1246–2341) were completed following established procedures (12–14).

Immunoprecipitation and Western Blotting. Cells were washed with PBS and trypsinized with 0.25% trypsin in Hanks' balanced salt solution. Equal numbers of cells were then washed three times with ice-cold PBS prior to lysis with 1 ml of lysis buffer [10 mM Tris (pH 7.5)-150 mM NaCl-1 mM EDTA-0.2% Nonidet P-40-1 μg/ml leupeptin and pepstatin A-1 mM phenylmethylsulfonyl fluoride]. Lysates were incubated on ice for 30 min and centrifuged at 16,000 × *g* for 15 min at 4°C. Supernatants were precleared with protein A-agarose (Oncogene Science, Inc.) and immunoprecipitated with monoclonal antibody 12CA5, on recognizing the HA epitope, on a rotating platform at 4°C for 3 h. Immunocomplexes were isolated by adding 15 μl of protein A-agarose and rotating for an additional 60 min at 4°C. After three washes with lysis buffer, samples were fractionated on SDS-polyacrylamide gels and electrophoretically transferred to nitrocellulose membranes using standard conditions. Membranes were blocked for 3 h at room temperature in PBS containing 5% dry milk, and 2.5% Tween 20, washed several times (5 min each) with washing buffer (PBS with 0.5% Tween 20), and incubated with primary antibody at room temperature for 2 h. After several 5-min washes with washing buffer, membranes were incubated for 2 h with goat anti-mouse immunoglobulin conjugated to horseradish peroxidase (Amersham). Membranes were then washed and developed using chemiluminescence substrates according to manufacturer's instructions (enhanced chemiluminescence mixture; Amersham).

Cell Culture and Transfection. TK-ts13 hamster fibroblasts (15) growing in Dulbecco's modified Eagle's medium with 10% fetal bovine serum were transfected by the standard calcium phosphate precipitation method (16).

CAT Assays. Two reporter constructs were used: (*a*) a minimal human c-*myb* promoter driving the *CAT* gene and containing three Myb-binding sites of the Myb responsive *MIM-1* gene (8) upstream of the minimal c-*myb* promoter (MIM-1-P1CAT); and (*b*) the *CD34-CAT* gene containing the 5′ flanking region (nt −293 to +55) of the Myb-responsive human CD34 promoter (9) linked to the *CAT* gene. Synthetic oligonucleotides including the Myb-binding sites of the *MIM-1* gene were first annealed to generate double-stranded oligomers, then phosphorylated with a T4 polynucleotide kinase, multimerized using T4 DNA ligase, purified by polyacrylamide gel electrophoresis, blunt-ended with the Klenow polymerase, and cloned upstream of the minimal c-*myb* promoter of the Myb-P1CAT construct.

CAT assays were performed as described (17). Briefly, TK-ts13 hamster fibroblasts were transfected with 1 μg of CAT reporter plasmid with or without 1 or 5 μg of effector plasmid plus 1 μg of DNA pol-β-gal, which contains the bacterial β-galactosidase gene driven by the DNA polymerase α-promoter as an internal control of transfection efficiency. At 48 h after transfection, cells were harvested and proteins were extracted by freeze-thawing and then normalized for transfection efficiency by β-galactosidase assay as described by the manufacturer (Promega Corp.). For each assay, cellular lysate was incubated with [^{14}C]chloramphenicol and acetyl-CoA for 1 h at 37°C. Transactivation of reporter constructs was assayed by measuring the amount of acetylated [^{14}C]chloramphenicol by thin-layer chromatography followed by autoradiography and Cerenkov counting.

RESULTS

Cloning and Expression of a Full-Length A-*myb* cDNA. To obtain a full-length A-*myb* cDNA, we screened a cDNA library derived from chronic lymphocytic leukemia cells using a 5′ segment of the human A-*myb* cDNA (clone A-*myb* 1). Several positive clones were isolated and sequenced to determine the identity with the published sequence of A-*myb* cDNA and to select the clone with the longest insert. Since none of the clones contained the full-length A-*myb* cDNA, we first linked the longest insert that was isolated from the cDNA library to a fragment of the A-*myb* cDNA amplified by the reverse transcriptase-PCR method with primers derived from the published sequence of the partial-length A-*myb* (1). To obtain the full-length coding sequence of A-*myb*, we screened a human genomic library to identify a genomic clone containing the remaining distal segment of A-*myb*. After subcloning and sequence analysis of a 1.3-kilobase *Hin*dIII fragment that hybridized to the most distal portion of the partial-length A-*myb* cDNA, we linked the region of overlap to our longest A-myb cDNA insert, which was only 22 nucleotides short of the complete coding sequence as revealed by the position of the first stop codon (Fig. 1). We show only the region of overlapping with the previously published A-*myb* cDNA sequence (1). To determine whether the cloned A-*myb* cDNA generates an A-myb protein of the expected size, we attached a HA epitope at the NH_2 terminus of A-*myb*, inserted the A-*myb* cDNA into the pSV-based expression vector, and transfected TK-ts13 hamster fibroblasts with the construct. Northern blot analysis revealed the exogenous A-*myb* mRNA in transfected cells (Fig. 2*A*), and use of anti-HA monoclonal antibody 12 CA5 allowed detection of a protein with the expected size for A-*myb* (Fig. 2*B*).

A-*myb* Encodes a DNA-binding Protein Interacting with Myb-binding Sites. To demonstrate that A-*myb* encodes a DNA-binding protein that interacts with Myb-binding sites, the full-length or the amino-terminal portion of A-*myb* (nt 90–760) was prepared as a bacterially expressed GST-fusion protein and assayed by gel retardation analysis for the ability to interact with Myb-binding sites identified in the c-*myb*-responsive genes *MIM-1* (8) and *CD34* (9). The full-length and truncated GST-A-myb fusion proteins reacted with the oligomer including one copy of the Myb-binding site (*Box A* of the MIM-1 promoter) (Fig. 3*A*). Abrogation of the interaction by 100-fold excess of the wild-type, but not of the mutated, unlabeled double-stranded oligomer containing the Myb-binding site demonstrated the

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2283  CTTATTATGACTGAACAAGCAAGAAGATATCTGAGTACTTACACAGCTACCAGTAGTACT
      LeuIleMetThrGluGlnAlaArgArgTyrLeuSerThrTyrThrAlaThrSerSerThr

2343  TCAAGAGCTCTCATACTGTAATTGTTATTAAAATTGATGATATGCCCCACTCCCTTACTG
      SerArgAlaLeuIleLeu***

2403  CAGTCTCTACTAAATTAGGTTGCAGTGAAATTTTTCTCAATTAGTTGTTTTTAAAGTTGT

2463  AAGATAGCCCTTTTAATACAGCATCTTTTTTCTATTCTATATAGTAGGCAGAAAGCTAGT

2523  AAGTCACTTAAGGGGTAGATAGTTTCATAGTTTATTTTTTAAGAGATGAGATTTTTAAAA

2583  ATTGTTTTTAAAGAACAAGATGGGAAAATAATAGAATGTTCATGGATTTCTAAAAGTAAA

2643  TTCTCATATATTTTCTTCACAAGATATATGTTGCTACTCTCTTGATGCTGCAGTTTTGTT

2703  ATAGATAGGTGTATGAGTATATATGATTTCTGAAATTAGTCTATGTATGGAAAGCACACA

2763  TGATTTTATGAAGTACTTTTGCCCATGTGCTGATTTACTTAGGCTACCATTTACAAAGAA

2823  ACACATTGAAAAGGAATTTAAAGGAAGGATAGAAAGTTGCACTACTAATTTTTTGTTTTT

2883  TTTTTCAGAAGCAGTAAAATTAACTACAGTGTTAAATGTATTTATTTGAGCATAGTACTG

2943  AAAACAAAAAGCATTCAAAAAAGAGTTTTTTCTTTATTAGTAAATAGTATTTTCTTAATC

3003  TCAGAGGAGCTGAGAGTTTTGTTGAATGTATTGTACAGTATGTAGGAGCAGGAGAACTTT

3063  GTAAATTGGAAAGAAGTCTGTTTTTATAATTTATTTTTATTTTTAAAGCTT 3116
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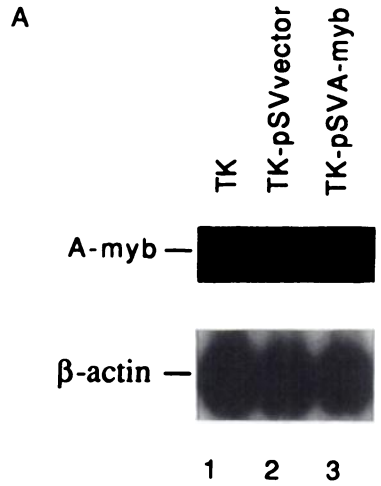
Fig. 1. Novel coding and 3′ untranslated sequence of A-*myb* cDNA. Nucleotide sequence extending that previously published is underlined.



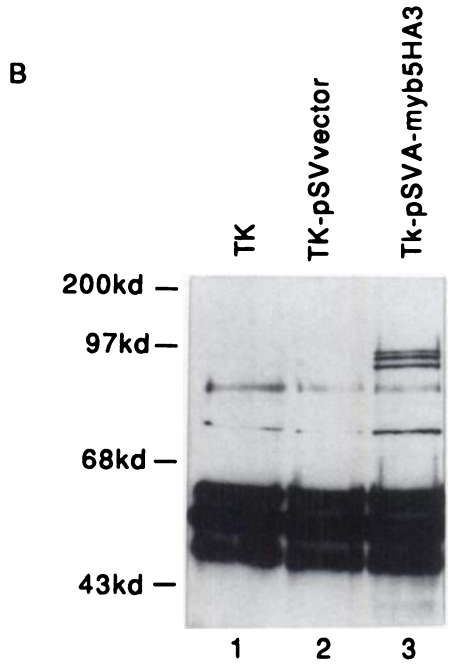


Fig. 2. A-*myb* expression in transiently transfected TK-ts13 cells. *A*, Northern blot analysis of A-*myb* mRNA levels in untransfected TK-ts13 cells (*Lane 1*) and in TK-ts13 cells 48 h after transfection with empty vector (*Lane 2*) or pSV-Amyb vector (*Lane 3*). *B*, immunoprecipitation and Western blot analysis of HA-tagged A-myb protein in transfected cells as detected with the 12CA5 anti-HA epitope monoclonal antibody. *Lanes* are the same as in *A*. *kd*, kilodalton.

specificity of the interaction (Fig. 3*A*). Similar experiments with an oligomer from the Myb-responsive human CD34 promoter containing two Myb-binding sites revealed specific interaction with bacterially synthesized GST-A-myb protein (Fig. 3*B*).

A-*myb* Transactivates Promoters Containing Myb-binding Sites. To determine whether the DNA-binding activity of A-*myb* is associated with negative or positive effects on promoters containing Myb-binding sites, we examined the ability of A-*myb* to transactivate CAT reporter constructs driven by a segment of the c-*myb* promoter (P1CAT; nt −78 to +203) linked to synthetic Myb-binding sites (*box A* of the MIM-1 promoter), or by a segment of the human CD34 promoter (nt −293 to +55). In TK-ts13 fibroblasts transfected at a 1:1 or 5:1 effector:reporter ratio and assayed for CAT activity 48 h later, the SV40-A-myb effector plasmid induced a 5–8-fold increase in CAT expression driven by the MIM-1 Myb construct (Fig. 4*A*) or by the CD34 construct (Fig. 4*B*).

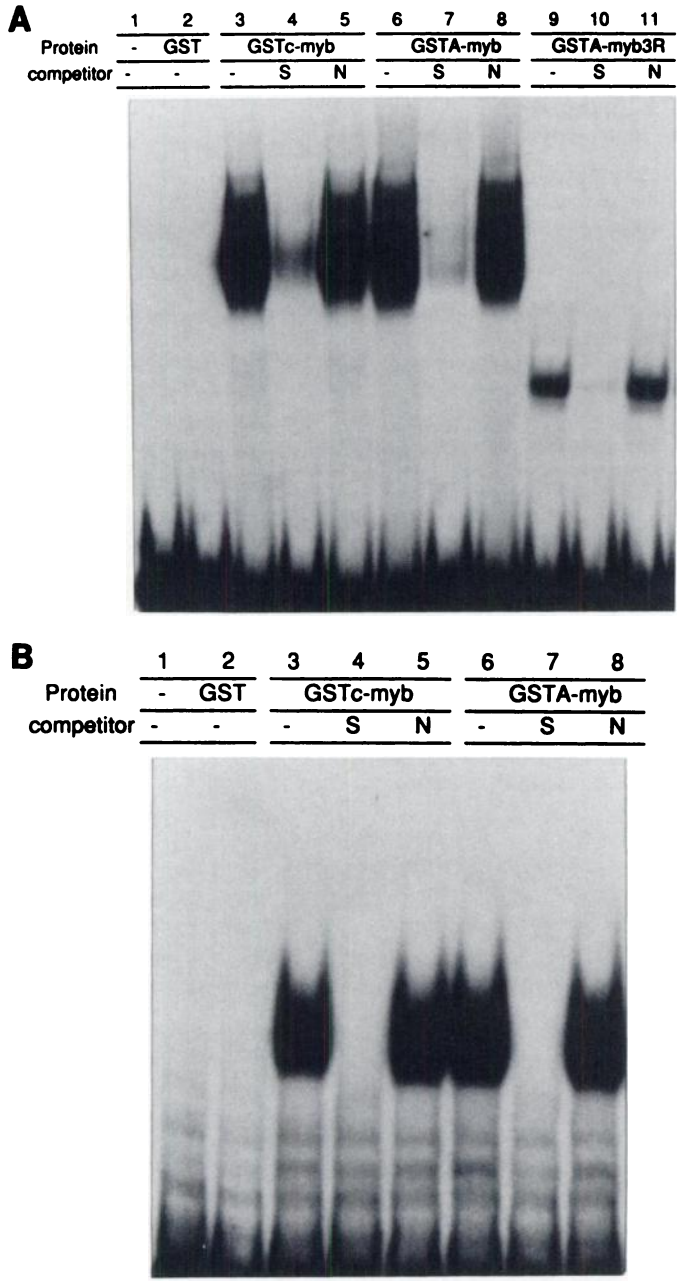


Fig. 3. A-*myb* binding to Myb binding sites. *A*, band-shift analysis of double-stranded oligonucleotides containing *Box A* of the MIM-1 promoter and the recombinant GST-A-myb proteins. GST A-myb 3R indicates GST plus the A-*myb* DNA-binding domain; *S*, specific competitor; *N*, nonspecific competitor. *Lane 1*, lysate only; *Lane 2*, GST protein only; *Lane 3*, GST-c-myb protein only; *Lane 4*, GST-c-myb protein in the presence of specific competitor; *Lane 5*, GST-c-myb protein in the presence of nonspecific competitor; *Lane 6*, GST-A-myb protein only; *Lane 7*, GST-A-myb protein in the presence of specific competitor; *Lane 8*, GST-A-myb protein in the presence of nonspecific competitor; *Lane 9*, truncated GST-A-myb protein only; *Lane 10*, truncated GST-A-myb protein in the presence of specific competitor; *Lane 11*, truncated GST-A-myb protein in the presence of unspecific competitor. *B*, band-shift analysis of double-stranded oligonucleotides corresponding to a Myb-binding site of the CD34 promoter and the recombinant GST-A-myb protein. *S*, specific competitor; *N*, nonspecific competitor. *Lanes* are as in *A*, except that experiments with truncated GST-A-myb protein were not performed.

DISCUSSION

We demonstrate here that A-*myb* encodes a DNA-binding protein with sequence specificity identical or similar to that of c-*myb* and B-*myb*. More important are the data indicating transactivation of two Myb responsive promoters (Fig. 4) which suggest a positive correlation between DNA-binding and transactivation activity. Transactivation of the MIM-1-P1CAT construct was also observed with two hybrid cDNAs containing either the c-*myb* DNA-binding domain linked to the putative transactivation domain of A-*myb* or the A-*myb* DNA-binding domain linked to c-*myb* transactivation domain (Fig. 4*A*). These observations raise the possibility that the functional domains of c-*myb* and A-*myb* are interchangeable and that the distinct function of these two genes may derive from their pattern of expression and/or their preferential interaction with Myb-binding sites of target genes.

In contrast to the reported decrease in A-*myb* mRNA levels following G_0 exit of peripheral blood lymphocytes by phytohemagglutinin stimulation (4), we find that A-*myb* mRNA levels increase on

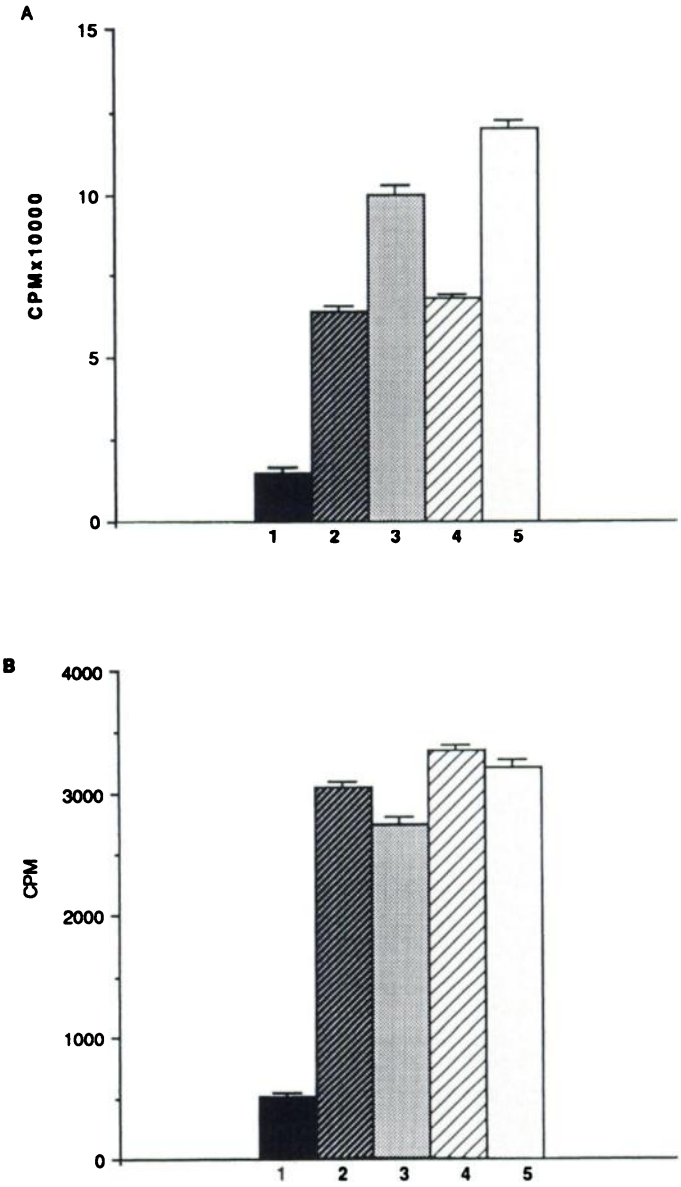


Fig. 4. Transactivation of c-*myb*-responsive promoters by pSV-A-*myb* in transient transfection assay. *A*, transactivation of the MIM-1 promoter. Histograms of CAT activity in lysates of TK-ts13 cells transfected with: 1 μg MIM-1-P1CAT (*Lane 1*); 1 μg MIM-1-P1CAT plus 1 μg pSV-A myb (*Lane 2*); 1 μg MIM-1-P1CAT plus 1 μg pSV-c-myb (*Lane 3*); 1 μg MIM-1-P1CAT plus 1 μg pSV-A-myb DB-c-myb (*Lane 4*); 1 μg MIM-1-P1CAT plus 1 μg pSV-c-myb DB-A-myb (*Lane 5*). *Bars*, mean + SD of three different experiments. *B*, transactivation of the CD34 promoter. Histograms of CAT activity in lysates of TK-ts13 cells transfected with: 1 μg CD34-CAT (*Lane 1*); 1 μg CD34-CAT plus 1 μg of pSV-A-myb (*Lane 2*); 1 μg CD34-CAT plus 5 μg of pSV-A-myb (*Lane 3*); 1 μg CD34-CAT plus 1 μg pSV-c-myb (*Lane 4*); 1 μg CD34-CAT plus 5 μg pSV-c-myb (*Lane 5*). *Bars*, mean + SD of three different experiments.

serum stimulation of T98G glioblastoma cells (not shown), suggesting that, at least in some cell types, the pattern of A-*myb* expression is similar to that of c-*myb* or B-*myb* (18–20). At present, it remains unclear whether A-*myb* and c-*myb* are functionally similar in nonhematopoietic cells expressing A-*myb* or whether the regulation of *Myb*-responsive genes in cells coexpressing A-*myb* and c-*myb* depends on the relative transactivating potency of each Myb family member. The availability of a full-length A-*myb* cDNA enables investigations of these possibilities and a detailed analysis of the significance of the interrelationships between A-*myb* and the other Myb family members in the regulation of cell proliferation, differentiation, and development.

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