# Human A-*myb* gene encodes a transcriptional activator containing the negative regulatory domains

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Received 6 December 1994

**Abstract** **The *myb* gene family has three members, c-*myb*, A-*myb*, and B-*myb*. A-*myb* mRNA is mainly expressed in testis and peripheral blood leukocytes. A-Myb can activate transcription from the promoter containing Myb-binding sites in all cells examined. In addition to the two domains (a DNA-binding domain and a transcriptional activation domain), two negative regulatory domains have been identified in A-Myb. These results indicate that A-Myb functions as a transcriptional activator mainly in testis and peripheral blood cells, and the regulatory mechanism of A-Myb activity is similar to that of c-Myb.**

*Key words:* A-*myb* gene; Tissue-specific expression; Transcriptional activator; Negative regulatory domain

## 1. Introduction

The *myb* gene was originally identified as a transforming oncogene of avian myeloblastosis virus and E26 leukemia virus [20,34]. The c-*myb* proto-oncogene, the cellular homolog of v-*myb*, is essential for the proliferation of hematopoietic progenitor cells. The level of c-*myb* expression is predominantly, although not exclusively, high in immature hematopoietic cells, and its expression is turned off during terminal differentiation [8,16,43]. Constitutive expression of introduced c-*myb* blocks the induced differentiation of immature erythroid cells [5,41], and treatment with an antisense oligonucleotide directed to c-*myb* blocks the proliferation of hematopoietic precursor cells [13]. Furthermore, homozygous c-*myb* mutant mice are severly anemic and die in utero due to defective fetal hematopoiesis [25]. In addition to the role in hematopoietic progenitor cells, c-*myb* is also important in the mitogen-induced proliferation of T lymphocytes and smooth muscle cells. c-*myb* expression is induced after mitogenic stimulation of both T lymphocytes and smooth muscle cells, in parallel to entry into the S phase [4,38]. Inhibition of c-*myb* function by a dominant negative form or antisense oligonucleotide blocks thymopoiesis or proliferation of carotid arterial smooth muscle cells [1,36].

The c-*myb* gene product (c-Myb) is a transcriptional activator that can bind to specific DNA sequences, AACNGN(A/T/C) [2,39,45]. Both artificial promoter constructs containing Myb-binding sites [26,30,44] and the natural promoters of *mim-1* and c-*myb* genes [9,27,29,46] are activated by c-Myb. c-Myb has three functional domains, responsible for DNA binding, transcriptional activation, and negative regulation, respectively [35]. The negative regulatory domain (NRD), located in the carboxyl-proximal portion of the molecule, is important for modulation of c-Myb activity. Deletion of this domain increases both transactivating and transforming capacities, implying that this domain normally represses c-Myb activity [17,35]. A leucine zipper motif is in this NRD, and its disruption also markedly increases c-Myb activity [18]. Since the leucine zipper motif is well known to mediate dimerization of several DNA-binding proteins, these observations suggest that an inhibitor binds to c-Myb through this leucine zipper.

We isolated two c-*myb*-related genes, A-*myb* and B-*myb*, from human DNA libraries by cross-hybridization with a c-*myb* DNA probe [31]. In contrast to the restricted expression of c-*myb* mainly in the immature hematopoietic cells, their expression is not restricted to the hematopoietic system [31]. Expression of both c-*myb* and B-*myb* are growth-regulated, and increase in the $G_1$ period of cell cylcle, reaching a peak at the $G_1$/S boundary [14,33]. In contrast, A-*myb* is expressed in resting T cells, and its levels gradually decrease after mitogenic stimulation [14]. These facts suggest that the physiological role of A-*myb* is distinct from that of c-*myb* and B-*myb*. Both A-Myb and B-Myb show extensive homology to c-Myb in three regions (the DNA-binding domain, the carboxyl-terminal half of the NRD, and the carboxyl-terminal short region), suggesting that they may also be transcription activators. We demonstrated that B-Myb functions as a transcriptional activator [24], and identified the transcriptional activation domain containing a cluster of acidic amino acids [28]. In contrast to our observation, other groups reported that B-Myb has no transcriptional activating capacity and functions as a repressor by competing with c-Myb for binding to DNA [11,42]. Our recent analyses to resolve this discrepancy indicate that B-Myb is a cell type-specific transcriptional activator (unpublished results).

To understand the physiological role of A-Myb compared with c-Myb and B-Myb, we have investigated the tissue specificity of A-*myb* expression and transcriptional activation by A-Myb. A-*myb* mRNA is mainly expressed in testis and peripheral blood cells. Like c-Myb, but not B-Myb, A-Myb functions as a transcriptional activator in all cells examined, and its activity is regulated by the NRD.

## 2. Materials and methods

### 2.1. Northern blotting

The human tissue blots were purchased from Clontech (Palo Alto). As the A-*myb*-specific DNA probe, the 0.6-kb *NdeI–NcoI* DNA fragment (nucleotides 745–1315; nucleotide numbers are as in [31]) was

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*SSDI* 0014-5793(94)01402-7

prepared from the human A-*myb* cDNA clone. The 2.0-kb *Nco*I–*Xba*I DNA fragment (nucleotides 113–2100; nucleotide numbers are as in [22]) prepared from the human c-*myb* cDNA clone was used to detect the c-*myb* RNA. Hybridization was done for 20 h at 42°C in the presence of 50% formamide 5 × SSPE, 2% SDS, 10 × Denhardt's solution, and 100 μg/ml of denatured salmon sperm DNA. The membranes were washed in 0.1 × SSC, 0.1% SDS at 50°C.

*2.2. Plasmid construction*

To isolate the cDNA clone encoding the C-terminal portion of the human A-Myb, which had been lacking in the previously characterized clones, the 3′ RACE (rapid amplification of cDNA ends) method was used as described by Frohman et al. [12]. Since analyses of the previously isolated cDNA clone showed that it lacked a very short region encoding 7 amino acids, a synthetic oligonucleotide containing this portion was ligated to the previously isolated cDNA clone to generate the cDNA clone encoding the full-length A-Myb. The plasmid pact-A-*myb* expressing the full-length A-Myb was constructed by use of the A-*myb* cDNA and the plasmid containing the 5′ regulatory region of the chicken β-cytoplasmic actin gene, as described in the case of pact-B-*myb* [24].

All plasmids designed to express mutant A-Myb proteins in cultured cells were generated from the plasmid pact-A-*myb*. A series of C-terminal truncated mutants of A-Myb were constructed by generating termination codons at the appropriate sites by oligonucleotide-directed mutagenesis as described by Kunkel et al. [21]. In mutants CT673, CT590, and CT296, an *Xba*I site that overlapped the termination codon was also generated. This caused a conversion of the Asn-673 in CT673 and Ser-296 in CT296 to Cys and Ile residues, respectively. Similarly, in CT396 and CT325, *Eco*RI and *Hpa*I sites were generated, respectively. In CT325, Asp-325 was converted to a Gly residue. To make the mutant ΔTA, lacking the region between amino acids 218 and 278, the *Nde*I–*Pvu*II fragment was deleted from pact-A-*myb* and then self-ligated after generating a flush end with Klenow enzyme.

*2.3. DNA transfection, CAT assay, and RNase protection analysis*

Co-transfection experiments using African green monkey kidney cells (CV-1), mouse fibroblasts (NIH 3T3), or human epitheloid carcinoma cells (HeLa) and the CAT reporter plasmid pA10CAT6MBS-I, were done as described [26]. All CAT co-transfection experiments were repeated three or four times, and typical results are shown. The difference between each set of experiments were within 30%. The RNase protection analysis was done as described [24,27].

*2.4. Western blot analysis of A-Myb mutants*

A mixture of 9 μg of the plasmid DNA to express A-Myb mutants and 0.1 μg of the internal control plasmid pact-β-gal was transfected using the $CaPO_4$ method into 293T cells (derived from the adenovirus type 5-transformed human embryonic kidney 293 cell line) [7]. A-Myb proteins were detected as described by Western blotting with the rabbit polyclonal antibody αCT5 raised against the fusion protein (GST-CT5) containing glutathione *S*-transferase (GST) and the c-Myb DNA-binding domain.

*2.5. Gel mobility shift assay by using lysates prepared from transfected cells*

The plasmid DNA to express various forms of A-Myb was transfected into 293T cells, and nuclear extracts (NE) were prepared 48 h after transfection as described by Dignam et al. [6]. The amount of A-Myb protein in NE was examined by Western blotting. The gel mobility shift assay was done as described [19].

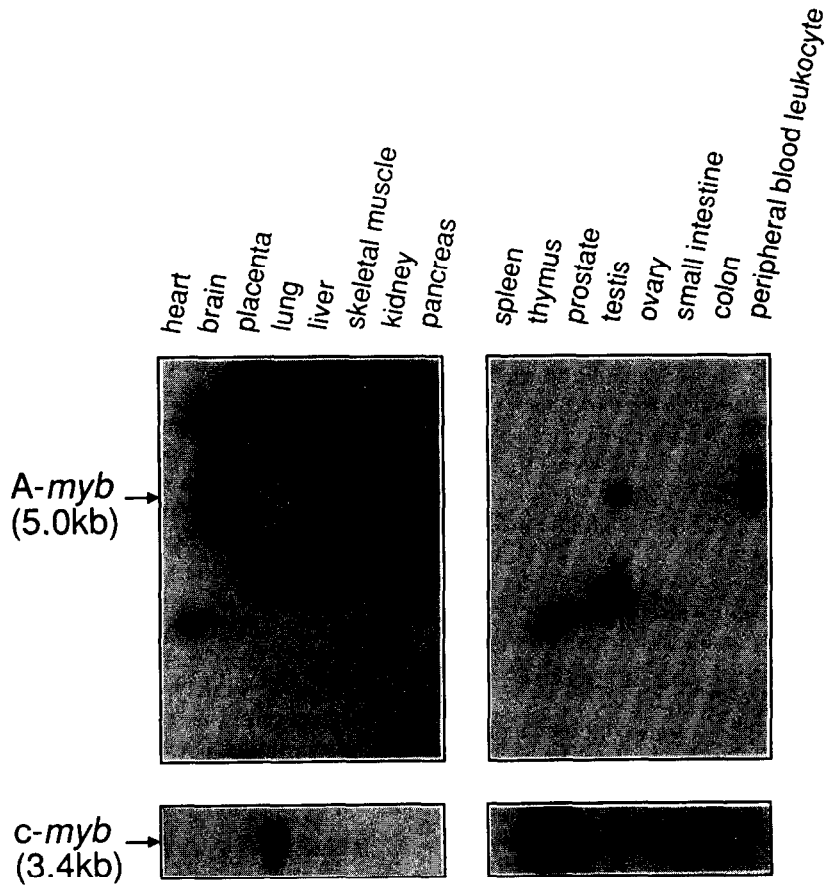


Fig. 1. Tissue distribution of A-*myb* mRNA in human adult tissues. RNA filters were obtained from Clontech (Palo Alto, CA), and each lane contained 2 μg of poly(A)$^+$ RNA. Northern blot analyses were done as described in section 2. The 5.0-kb A-*myb* mRNA and 3.4-kb c-*myb* mRNA are indicated by arrows.

## 3. Results

### 3.1. Tissue distribution of A-myb mRNA

To examine the pattern of expression of A-*myb* in various human tissues, we used the A-*myb*-specific DNA probe in Northern blot analysis. As shown in Fig. 1 5.0-kb A-*myb* mRNA was detected only in testis and peripheral blood leukocytes. The sequential hybridization of the same blots with the c-*myb*-specific probe indicated that c-*myb* has a distinct pattern of expression from A-*myb* (Fig. 1). The 3.4-kb c-*myb* mRNA is expressed at the highest level in thymus, at a moderate level in lung, small intestine, and colon, and at a low level in prostate, ovary, and peripheral blood leukocytes. Thus, the tissue specificity of expression is clearly different between A-*myb* and c-*myb*.

### 3.2. Transcriptional activation by A-Myb

We had isolated and characterized the human A-*myb* cDNA clones, but they lacked the C-terminal portion of the A-Myb-coding region [31]. To analyze the function of A-Myb, it is necessary to isolate the cDNA clones encoding the full-length A-Myb. By using the 3′ RACE method, we have isolated a cDNA clone encoding the C-terminal region of A-Myb. Structure analyses of this clone indicated that the full-length A-Myb contains 7 more amino acids (TSRALIL) in its C-terminal region than the previously reported protein [31]. Based on this finding, we have constructed an expression vector to express the full-length A-Myb.

To examine whether A-Myb is also a transcriptional activator like c-Myb, we did a co-transfection experiment with the A-*myb* expression plasmid pact-A-*myb* and the reporter plasmid pA10CAT6MBS-I (Fig. 2A). This reporter plasmid contains six tandem repeats of the MBS-I sequence, to which both c-Myb and B-Myb can specifically bind, linked to the SV40 early promoter and the CAT gene. We have also confirmed that A-Myb can bind to the MBS-I sequence (see Fig. 5). We had reported that in a co-transfection assay with increasing amounts of effector plasmid, the c-Myb activity increases up to a maximum with about 0.75 μg of c-Myb expression plasmid, and then decreased with further increases in the amount of c-Myb [32] (also see Fig. 2A). This negative autoregulation is a consequence of c-Myb homodimer formation through the leucine zipper and the inability of the c-Myb homodimer to bind to DNA. In contrast, B-Myb activity is not autoregulated by a similar mechanism [28] (also see Fig. 2A). In CV-1 cells, the degree of transactivation by wild-type A-Myb increased up to a maximum 8-fold with 2 μg of A-Myb expression plasmid. After reaching this maximum, the degree of transactivation decreased with further increases in the amount of A-Myb. These results indicate that A-Myb can function as a transcriptional activator and also that A-Myb activity is negatively regulated like c-Myb.

To confirm that A-Myb activated transcriptional initiation, we did S1 nuclease mapping using the reporter plasmid pA10MβG6MBS-I, in which the CAT gene of pA10CAT6MBS-I was replaced by the mouse β-globin gene (Fig. 2B). As expected, the protected DNA fragment of 186–190 nucleotides, which corresponds to the mouse β-globin transcripts started at the correct site, was detected in transfections containing pact-A-*myb*, although the levels of β-globin mRNA in transfections containing the control effector plasmid were almost undetectable.

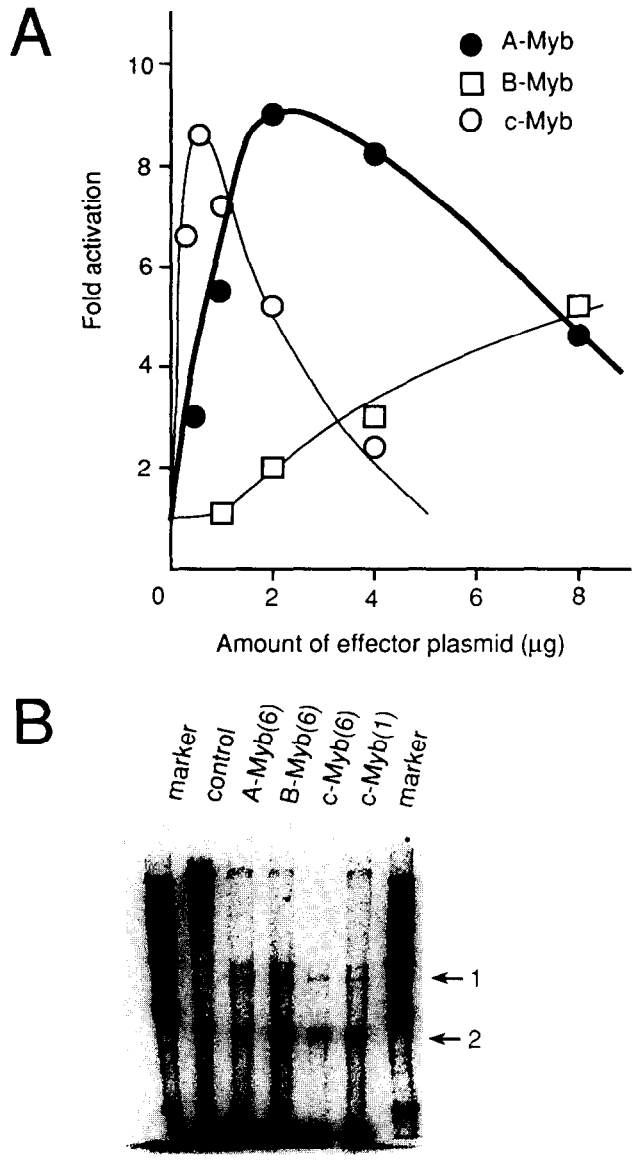


Fig. 2. Transcriptional activation by A-Myb. (A) CAT co-transfection experiments. Mixtures of 4 μg of the reporter plasmid pA10CAT6MBS-I DNA, varying amounts of the effector plasmid DNA to express A-Myb, B-Myb, or c-Myb, and 2 μg of the internal control plasmid pact-β-gal DNA were transfected into CV-1 cells. The total amount of effector plasmid was adjusted to 8 μg by addition of varying amounts of pact-1 DNA. CAT assays were done for 1.5 h. The degree of transactivation (compared with a sample without Myb expression plasmid) was measured and is shown. (B) RNase protection analysis. Mixtures of the reporter plasmid pA10MβG6MBS-I DNA (6 μg), the effector plasmid DNA (6 μg) to express A-Myb, B-Myb, c-Myb, or no protein (control), and the reference plasmid pRSV-MβG DNA (2 μg) were transfected into CV-1 cells, and RNA was prepared 40 h after transfection. An RNase protection assay was done with 20 μg of total RNA. The protected fragments that correspond to transcripts initiated at the SV 40 early promoter in pA10MβG6MBS-I and the RSV promoter in pRSV-MβG are indicated by arrows 1 and 2, respectively. $^{32}$P-Labeled pBR322 digested with *Hin*fI was used as a size marker.

B-Myb stimulates the CAT expression from the reporter plasmid pA10CAT6MBS-I in DV-1 and HeLa cells, but not in some other types of cells such as NIH 3T3 ([24], our unpublished results), indicating that B-Myb is a cell type-specific transcriptional activator. To examine whether A-Myb also

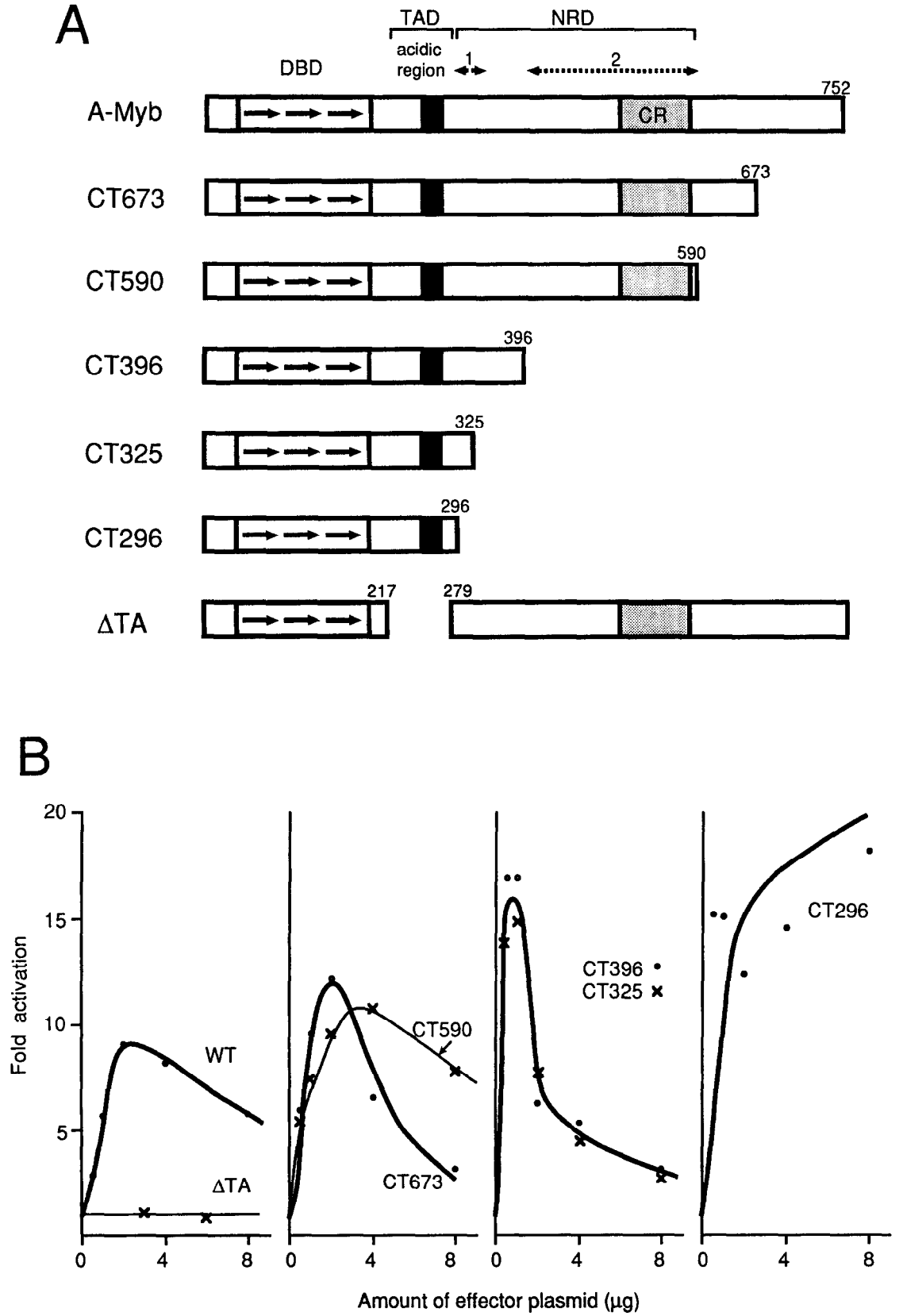


Fig. 3. Transactivation by A-Myb deletion mutants. (A) Structures of a series of deletion mutants. The three-fold tandem repeats in the DNA-binding domain (DBD) are indicated by the arrows. A closed box shows the region rich in acidic amino acids. The region conserved between three members of the *myb* gene family (CR) is indicated by the shaded box. (B) The results of CAT co-transfection experiments. CAT co-transfection experiments were done by using varying amounts of the effector plasmid DNA to express A-Myb mutant proteins indicated in each column as described in the legend to Fig. 2A. The degree of transactivation is shown.

does not have transactivating capacity in some types of cells like B-Myb, we used NIH 3T3 and HeLa cells for co-transfection experiments. A-Myb stimulated the CAT expression from the reporter plasmid pA10CAT6MBS-I about 7- and 5-fold in NIH 3T3 and HeLa cells, respectively (data not shown). We also confirmed that A-Myb also functions as a transcriptional activator in 293T cells in which B-Myb does not have the transactivating capacity (data not shown). Thus, our results indicate that A-Myb is a transcriptional activator in all the types of cells examined, like c-Myb.

*3.3. Deletion analyses of A-Myb*

To analyze the functional domains of A-Myb, we first made a deletion mutant of the putative transcriptional activation domain (ΔTA), which is rich in acidic amino acids and located in the region downstream of the DNA-binding domain (Fig. 3A). As expected, the ΔTA mutant did not stimulate the CAT expression from the pA10CAT6MBS-I reporter (Fig. 3B), indicating that this acidic region functions as a transcriptional activation domain. To examine whether A-Myb also has a regulatory domain like the NRD of c-Myb, a series of carboxyl-terminally truncated mutants was constructed (Fig. 3). To identify the regulatory domain of A-Myb clearly, we measured transactivation by various forms of A-Myb with increasing amounts of A-Myb expression plasmid (Fig. 3B); we have verified that this results in correspondingly varying amounts of protein in the transfected cells (data not shown). The transactivation by increasing amounts of two C-terminal mutants CT590 and CT678 were similar to that of wild-type, indicating that the C-terminal 152-amino acid region does not affect the A-Myb activity. Further truncation to amino acids 396 or 325 changed the dose–response curve. The degree of transactivation by CT396 or CT325 increased to a maximum 15- to 17-fold, which is about 2-fold higher than the wild-type, with 0.5 μg of A-Myb expression plasmid, and then turned off rapidly with further increases in the amount of effector plasmid. These results suggest that the region between amino acids 396 and 596 is the regulatory domain, deletion of which enhances the transactivation by low amounts of A-Myb but does not affect the negative regulation by increasing amounts of A-Myb. In contrast, the degree of transactivation by CT296 increased continuously with the amount of A-Myb expression plasmid. Thus, the degree of transactivation with 8 μg of the A-Myb expression plasmid was 18-fold by CT296, but only 2.7-fold by CT325. These results indicate that the small region between amino acids 296 and 325 is essential for the negative regulation of A-Myb activity. Thus, two NRDs, amino acids 396–596 and 296–325, were identified in A-Myb.

To examine whether the expected size of proteins were produced from each expression plasmid and whether the deletion mutations affected the protein stability, we analyzed the mutant proteins expressed in 293 T cells after DNA transfection (Fig. 4). The wild-type and six mutant proteins were detected by Western blotting as the expected size of proteins by using the polyclonal antibody αCT5 raised against a fusion protein comprising GST and the DNA-binding domain of c-Myb. All of these proteins were expressed at almost the same level, indicating that these six mutant proteins also had similar stability.

*3.4. Negative regulation of DNA-binding activity of A-Myb*

To examine whether the DNA-binding activity of A-Myb is

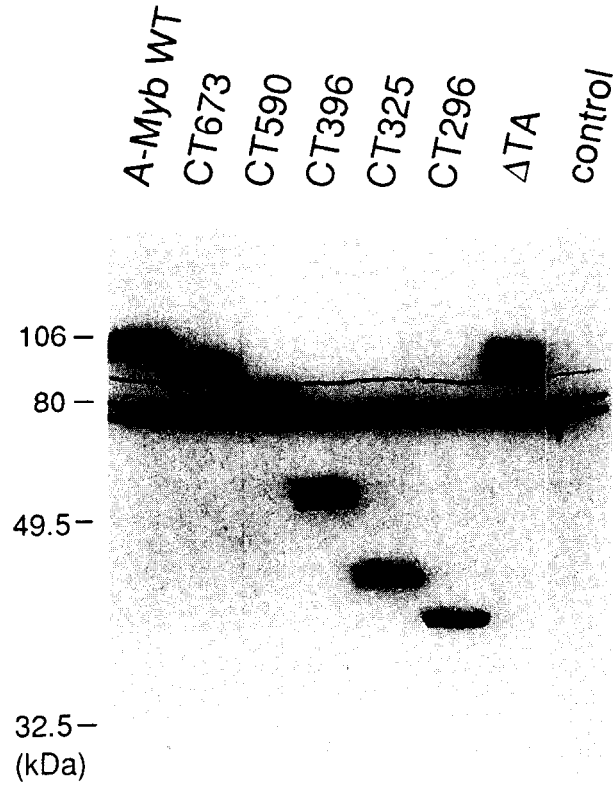


Fig. 4. Immunodetection of A-Myb mutant proteins expressed in transfected cells. A mixture of 9 μg of DNA to express the A-Myb proteins indicated above each lane or no protein (control) and 0.1 μg of the internal control plasmid pact-β-gal DNA were transfected into 293T cells. Proteins were prepared from transfected cells, separated by 10% SDS-PAGE, and then the proteins were detected by Western blotting with polyclonal antibody αCT5. Prestained molecular size markers (Bio-Rad) are indicated to the left.

negatively regulated by a specific region, especially the NRDs described above, we did gel mobility shift experiments using NE that was prepared from 293T cells transfected by the expression plasmid encoding various forms of A-Myb (Fig. 5). The DNA binding of Myb was not detected by using NE prepared from transfected CV-1 or NIH 3T3 cells; probably the efficiency of transfection into these cell lines were low. The cell line 293T is more transfectable than these cells, and the DNA binding activity of c-Myb can be easily detected by using NE prepared from the transfected 293T cells (our unpublished results). When we used NE prepared from 293T cells transfected by wild-type, CT678, or CT590 expression vectors, no specific retarded band corresponding to the A-Myb–DNA complex was detected. In contrast to this, NE containing CT396, CT325, or CT296 generated specific retarded bands. To confirm that the generated band contains the A-Myb protein expressed from the transfected DNA, the Myb-specific antibodies were added to the binding reaction. Among three antibodies used, the monoclonal antibody MAb5-1, which can recognize both c-Myb and A-Myb, supershifted the retarded band, indicating that the observed retarded band was generated by A-Myb. These results indicate that the DNA-binding activity of A-Myb is negatively regulated by the region between amino acids 396 and 590, and that the removal of this region increases the DNA binding. These results are consistent with the results of CAT co-transfec-

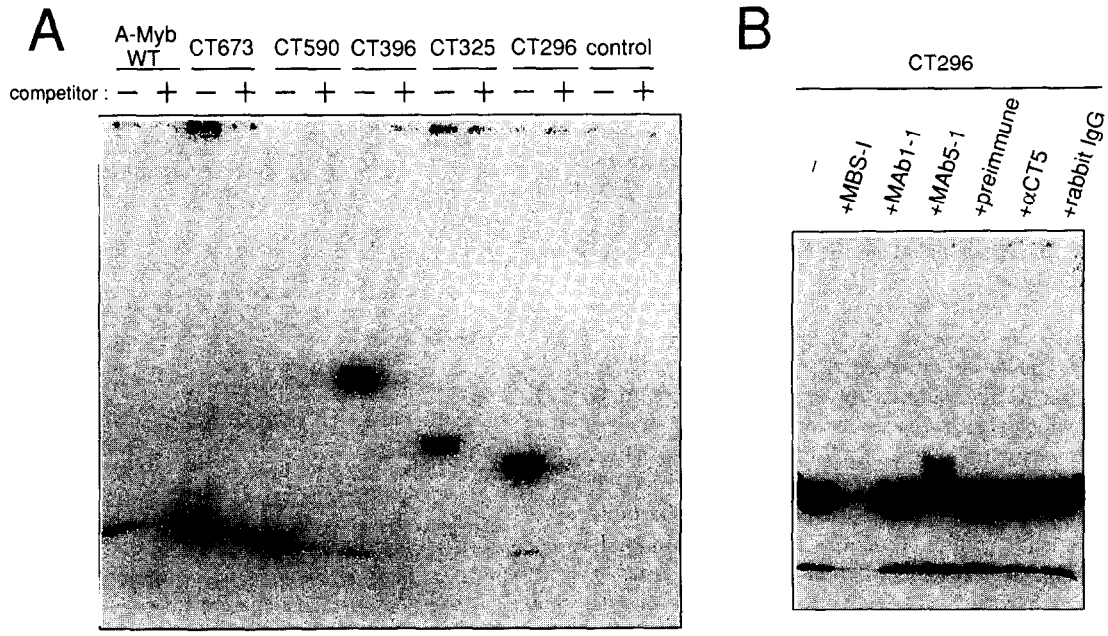


Fig. 5. Negative regulation of the DNA-binding activity of A-Myb by the C-proximal portion. (A) Gel retardation analysis using NE prepared from 293T cells transfected by A-Myb expression plasmid. 293T cells were transfected by the plasmid to express various forms of A-Myb shown above each lane, or no protein (control), and NE was prepared. The probe containing the Myb-binding site, MBS-1, was used for the binding reaction. In lanes indicated by +, a 100-fold molar excess unlabeled DNA competitor containing the MBS-1 sequence was added. (B) Effects of the Myb-specific antibodies on the formation of the DNA-protein complex. The gel shift assays were done using the MBS-1 probe and NE that was prepared from 293T cells transfected by A-Myb CT296 expression plasmid. The anti-c-Myb monoclonal antibodies (MAb1-1 and MAb5-1), polyclonal antibody raised against the DNA-binding domain of c-Myb (αCT5), rabbit normal serum (preimmune), purified rabbit IgG, 100-fold molar excess unlabeled DNA competitor containing the MBS-1 sequence (MBS-1), or no competitor (−) were added to the binding reaction.

tion experiments that the degree of transactivation by CT396 is about 2-fold higher than wild type with a small amount of A-Myb expression plasmid.

## 4. Discussion

The c-*myb* mRNA was originally found to be expressed predominantly in immature hematopoietic cells. However, it was reported that more tissues and cells expressed the c-*myb* mRNA as shown in Fig. 1 [40]. The cell type and tissue specificity of expression of the B-*myb* mRNA is broader than that of c-*myb* (our unpublished results). Compared with c-*myb* and B-*myb*, the tissue specificity of A-*myb* expression is narrow and restricted to testis and peripheral blood leukocytes (Fig. 1). The expression of A-*myb* mRNA in testis was also reported in *Xenopus* and mouse [3,23,37]. The *Xenopus* and mouse A-*myb* expression is observed through the early stages of sperm development during the proliferation of spermatogonial cells, and then down-regulated as the primary spermatocytes meiotically divide [23,37]. Such expression has strong parallels with c-*myb* expression in immature haematopoietic lineages. However, in contrast to c-*myb* and B-*myb*, human A-*myb* is expressed in resting T lymphocytes, and its levels gradually decrease after mitogenic stimulation [14]. Thus, expression of A-*myb* in testis, but not in T lymphocytes, appears to correlate with proliferation. To clarify the physiological role of A-Myb, it is important to clarify the relationship between A-*myb* expression and cellular proliferation.

Our analyses indicate that A-Myb functions as a transcriptional activator in all the cells examined. During preparation of this manuscript, two other groups also reported that A-Myb is a transcriptional activator [10,15]. This characteristic is the same as c-Myb, but different from B-Myb, which is a cell type-specific transcriptional activator. The structures of functional domains of A-Myb, B-Myb, and c-Myb are shown in Fig. 6 and compared to each other. The structure of functional domain in A-Myb is very similar to c-Myb: DNA-binding domain, transcriptional activation domain, and negative regulatory domain from the N-terminus. In addition to the DNA-binding domain, the amino acid sequence in the transcriptional activation domain, especially the acidic region between amino acids 259 and 281 of A-Myb, is highly conserved between c-Myb and A-Myb. In contrast, the amino acid sequence in the corresponding region of B-Myb does not have significant homology with c-Myb and A-Myb. This difference may explain why A-Myb and c-Myb are transcriptional activators in all cells examined, but B-Myb is a cell type-specific transcriptional activator.

The results of our deletion analyses using a series of A-Myb deletion mutants indicate the presence of two NRDs in A-Myb: amino acids 396–590 and 296–325. The full-length form of A-Myb exhibited no DNA-binding activity in our gel mobility shift assay, but a deletion of the C-proximal region (amino acids 396–590) increased the DNA-binding capacity (Fig. 5A). Similar observations were also reported for several other DNA-binding proteins such as Ets. This probably causes an increase in transactivating capacity at low amounts of A-Myb. This C-proximal NRD contains the region conserved in the *myb* gene family. We have found that the conserved region in c-Myb negatively regulates its DNA-binding activity (our unpublished

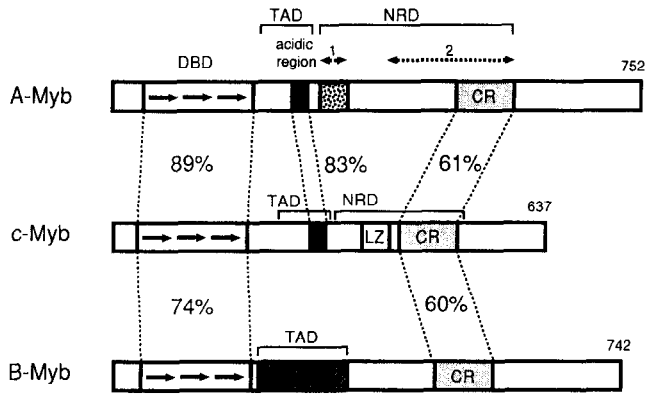


Fig. 6. Functional domains identified in A-Myb. Comparison of functional domains between three members of the *myb* gene family. The three tandem repeats in the DNA-binding domain (DBD) is shown by arrows. The closed box indicates the region that is rich in acidic amino acids. Two regions conserved between three members (CR) are indicated by shaded boxes. DBD, DNA-binding domain; TAD, transcriptional activation domain; NRD, negative regulatory domain; LZ, leucine zipper structure. Percentages indicate percent identity in each encompassed by dashed lines between human A-Myb and human c-Myb, or between human B-Myb and human c-Myb.

results). Deletion of this conserved region of c-Myb results in oncogenic activation of c-*myb*, and v-Myb carried by AMV lacks this region [17], suggesting that the corresponding region in A-Myb also has biological importance.

The transactivating capacity of A-Myb is also negatively regulated through the small region between amino acids 296 and 325. In co-transfection assays, maximal Myb-induced transactivation occurs with low amounts of wild-type A-Myb, while higher levels of A-Myb result in reduced A-Myb-induced transactivation. This negative regulation is impaired by a deletion of the region between amino acids 297 and 325. These observations are very similar to those obtained by c-Myb. c-Myb activity is also negatively regulated, and a disruption of the leucine zipper structure completely eliminated this regulation [32]. This negative regulation is due to the formation of the c-Myb homodimer through the leucine zipper and the inability of the dimer to bind to DNA, indicating that c-Myb itself can function as an inhibitor of c-Myb. Recently, we found that co-expression of the catalytic subunit of cAMP-dependent protein kinase (PKA) or the activated *src* gene product (Src) abrogates this negative regulation of c-Myb activity (our unpublished results). These results suggest that the specific growth signals that are transduced via PKA and Src, stimulates c-Myb activity through blocking the association of c-Myb with an inhibitor. The mechanism of negative regulation of A-Myb activity is unclear, but this may be important for the regulation of A-Myb activity by specific growth signals like in the case of c-Myb. The small region between amino acids 297 and 325 of A-Myb is predicted to form an amphipathic $\alpha$-helix and contains characteristic hydrophobic residues at every seventh position (leucine zipper-like motif). However, disruption of this leucine zipper-like motif by introducing point mutations did not abolish the negative regulation of A-Myb activity. Another type of structure in this region may be important for the negative regulation of A-Myb activity.

It should be noted that the amino acid sequence of the leucine zipper-containing region of c-Myb (amino acid 375–403) is significantly conserved in A-Myb (amino acids 341–369) which is located further downstream from the leucine zipper-like motif described above: 10 residues out of 29 amino acids are identical and 7 residues are similar. Although this region contains the putative leucine zipper motif, one proline residue is in this region, and a deletion of this region did not increase the transactivating capacity (our unpublished results). In addition, the corresponding region in *Xenopus* A-Myb contains three prolines, in spite of the highly conserved amino acid sequence between human A-Myb and *Xenopus* A-Myb in the whole protein-coding region. These results suggest that the amino acid sequence of the leucine zipper-containing region in c-Myb is considerably conserved in A-Myb, but its ability to bind to the inhibitor(s) was lost during evolution. Instead of this region, another region (amino acids 297–325) in A-Myb could obtain the capacity to interact with inhibitor(s), although this region does not have completely similar characteristics to the canonical leucine zipper motif.

Further analyses of the mechanism of negative regulation of A-Myb activity, and the identification of target genes in testis and peripheral blood leukocytes, the transcription of which is activated by A-Myb, will be important to understand the physiological role of A-Myb.

*Acknowledgements*: We are grateful to Dr. Tetsuya Sawazaki for preparation of the antibody $\alpha$CT5, and Dr. Takahiro Nagase for help with cloning of the A-*myb* cDNA clone.

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