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Individual Repeats of *Drosophila* Myb Can Function in Transformation by v-Myb

XU BIN AND JOSEPH S. LIPSICK†*

Department of Microbiology, State University of New York, Stony Brook, New York 11794-5222

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The v-Myb protein binds to specific DNA sequences and can regulate gene expression. The DNA-binding domain of v-Myb contains the second and third of the three highly conserved tandem repeats found in c-Myb. In general, the ability of mutant forms of v-Myb to transform correlates with their ability to *trans* activate transcription. Two mutations within the DNA-binding domain of v-Myb which preserve DNA binding in vitro but fail to *trans* activate or transform have been described. These results suggested that this highly conserved domain might function in specific protein-protein interactions, as well as in DNA binding. We therefore tested the ability of a related protein domain from *Drosophila melanogaster* to substitute functionally for the homologous region of v-Myb. We found that either the second or third repeat of *Drosophila* Myb, but not both, could function in *trans*-activation and transformation by v-Myb. The hybrid containing both the second and third repeats of *Drosophila* Myb bound to DNA but failed to *trans* activate transcription either in the context of v-Myb or as a v-Myb–VP16 fusion protein. These results demonstrate that although the protein-DNA contacts made by the Myb repeats have been conserved during the evolution of animals, the protein-protein interactions have diverged.

The v-*myb* oncogene of the avian myeloblastosis virus causes monoblastic leukemia in chickens and transforms myelomonocytic cells in culture (33). The protein it encodes can also cooperate with basic fibroblast growth factor to promote the growth of chicken neuroretinal cells in culture (10). The v-Myb protein is nuclear, binds to specific DNA sequences, and can regulate the expression of various reporter genes (1, 2, 26). v-Myb is a 48-kDa doubly truncated form of the normal 75-kDa c-Myb protein (11, 40). One or both of these truncations appear to be required for oncogenic activation of c-Myb (6, 13, 15). v-Myb has an N-terminal DNA-binding domain, a centrally located hydrophilic transcriptional activation domain, and an additional C-terminal domain that is required for transformation and for transcriptional activation in animal cells but not in *Saccharomyces cerevisiae* (5, 17, 19, 22, 25, 43, 51).

The amino terminus of v-Myb contains two of the three tandem repeats of approximately 50 amino acids that are present within the DNA-binding domain of c-Myb (14). The Myb repeats have no clear homology to other known DNA-binding motifs, although similarities to the helix-turn-helix structure of the homeodomain proteins and the basic region of the bZIP proteins have been proposed (3, 8, 36). These Myb repeats have been highly conserved during evolution, and Myb-related DNA-binding domains with 65% amino acid identity to vertebrate c-Myb have been identified in the invertebrate *Drosophila melanogaster* and the cellular slime mold *Dictyostelium discoideum* (23, 47). Both the *Drosophila* and *Dictyostelium* Myb proteins can bind to DNA sequences recognized by v-Myb (38, 47). More distantly related Myb DNA-binding domains have also been identified in green plants, including *Zea mays* (45% identity) and the budding yeast *S. cerevisiae* (30% identity) (37, 50).

Studies of mutant forms of v-Myb have implied that transformation of myelomonocytic cells correlates with the ability to *trans* activate transcription (28). Two mutant forms of v-Myb with altered N-terminal repeats failed to *trans* activate or transform while retaining wild-type levels of DNA binding in vitro (16, 28). One of these mutant proteins contained a two-amino-acid insertion, and the other contained a single amino acid substitution within the second Myb repeat. These results suggested that the highly conserved Myb repeats have a function in transcriptional activation in addition to their role in DNA binding. However, in the absence of structural data it is difficult to determine whether such site-directed mutations result in specific defects of protein function or more general defects in protein folding. Because the process of evolution continually selects for maintenance of proper protein folding and function, we chose to use the highly conserved repeats of *Drosophila* Myb as naturally occurring mutants with which to dissect the function of the homologous domains of v-Myb further. Therefore, we constructed hybrid proteins in which one or both of the highly conserved repeats of v-Myb were replaced by the corresponding repeats of *Drosophila* Myb. These hybrid proteins have been analyzed for the ability to transform myelomonocytic cells, to *trans* activate transcription, and to bind to DNA.

MATERIALS AND METHODS

Recombinant DNA. Retroviral expression vectors N-dGE, N-VVP, and pMAV-dX and bacterial expression vector MT7-dGE have been described previously (6, 30, 47). The region of *Drosophila myb* encoding residues homologous to the DNA-binding domain of v-Myb was constructed by annealing and ligating eight overlapping oligonucleotides, each approximately 80 nucleotides long, that were synthesized by Tracy Lane with a Pharmacia Gene Assember. This synthetic DNA contained a *Kpn*I restriction site at its 5′ end homologous to that of a previously described variant of v-*myb* (dGE) which lacks both *gag*- and *env*-encoded termini. The 3′ end of this DNA contained an *Eco*RI site which is shared between *Drosophila myb* and v-*myb*. This DNA was first cloned into the

* Corresponding author.
† Present address: Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305-5324.

unique *Kpn*I and *Eco*RI sites of pUC-MYB-dE (18). DNA sequencing revealed a single mutation predicted to cause an amino acid substitution. This mutation was repaired by site-directed mutagenesis (27). The *Kpn*I-*Bst*EII-resistant fragment encoding the *Drosophila* Myb DNA-binding domain was then transferred into MT7-dGE for bacterial protein expression. To conveniently separate the *Drosophila* Myb repeats, a silent *Hin*fI site homologous to that at nucleotide 165 of v-*myb* was created by site-directed mutagenesis by using a primer with the following sequence: TATTCGGATTCAGGTGGTTG (27, 42). After DNA sequencing, the second or third repeat of Drosophila Myb was transferred into v-Myb as a *Kpn*I-*Hin*fI or *Hin*fI-*Bst*EII-resistant DNA fragment, respectively. All constructions were confirmed by DNA sequencing. The hybrid *myb* genes were transferred into the N-dGE retroviral expression vector as *Kpn*I-*Cla*I-resistant DNA fragments. The corresponding Myb-VP16 expression vectors were constructed by transfer of *Eco*RI-resistant fragments into N-VVP.

Cell culture. The fibroblastic QT6 quail cell line was grown in Dulbecco modified Eagle medium supplemented with a high concentration of glucose (4.5 g/liter), 5% fetal bovine serum, 5% heat-inactivated chicken serum (1 h at 56°C), 2 mM sodium glutamine, 1 mM sodium pyruvate, 1× nonessential amino acids, penicillin (100 U/ml), and streptomycin (100 μg/ml). Cells were maintained at 37°C in a humidified incubator containing 10% CO_2–90% air.

Transfections and immunoblotting. Plasmids DNAs were transfected by a modified calcium phosphate method (4). Cells were washed and scraped in phosphate-buffered saline and then analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting as previously described (19). Myb proteins were detected with a mixture of monoclonal antibodies myb2.2 and myb2.7, both of which are directed against epitopes of v-Myb that lie C terminal to the conserved repeats (7). The blots were developed by incubation with alkaline phosphatase-coupled rabbit anti-mouse immunoglobulin (Promega) and then 5-bromo-4-chloro-3-indolylphosphate toluidinium (salt)–nitroblue tetrazolium.

Transformation assays. Transformation assays were performed with yolk sac cells from 12-day chicken embryos as described previously (18, 29). In brief, QT6 cells were transfected with 5 μg of *neo-myb* proviral DNA and 1 μg of MAV-1 helper virus DNA. Two days later, QT6 cells were selected for *neo* expression with 100 μg of G418 (Geneticin; GIBCO) per ml. The titer of the virus produced by these cells was determined by infection of fresh QT6 cells followed by selection with G418. Adherent, virus-producing QT6 cells were then treated with mitomycin C (10 μg/ml for 2 h), washed, and then used as feeder layers for infection of myelomonocytic precursor cells that were freshly isolated from the yolk sacs of 12-day chicken embryos. After 24 h of cocultivation, the nonadherent yolk sac cells were replated in fresh culture dishes. Cultures were fed with fresh medium every 3 to 5 days and monitored for the outgrowth of transformed cells. Genomic DNA from the transformed cells was analyzed by polymerase chain reaction (20). The primers used had the sequences GATTACCCA TGGC and GGAAGCCGGTGGTTGCC. DNA was denatured at 95°C for 5 min, and then amplification was performed for 25 cycles of the following sequence: 94°C for 30 s, 55°C for 60 s, and 72°C for 120 s.

Assays of CAT activity. QT6 cells were transfected with the indicated activator plasmid DNA (5 μg), either E1B-CAT or KHK-E1B-CAT reporter plasmid DNA (1 μg), and cytomegalovirus–β-galactosidase plasmid DNA (0.5 μg), which was used as an internal control for transfection efficiency (19). Two days after transfection, cells were washed and scraped in phosphate-buffered saline. Lysates were prepared by three cycles of freezing-thawing in 250 mM Tris-Cl (pH 8.0), and nuclei were pelleted by centrifugation. Supernatants were assayed spectrophotometrically for β-galactosidase activity (44). Normalized lysate samples were then assayed for chloramphenicol acetyltransferase activity by the phase extraction method (45).

Bacterial protein expression. Bacterial expression plasmids were transferred into the BL21 (DE3) (LysS) host strain, kindly provided by F. W. Studier (Brookhaven National Laboratory, Upton, N.Y.). Bacteria were grown in M9-ZB supplemented with ampicillin (50 μg/ml) and chloramphenicol (30 μg/ml) at 30°C (49). When the optical density at 600 nm reached 0.4 to 0.7, isopropyl-β-D-thiogalactopyranoside (0.5 mM) was added and the incubation was continued for another 5 h at 37°C. Bacteria were pelleted by centrifugation, and lysates were prepared by freezing-thawing in buffer containing 6 M urea as previously described (9). Protein expression was monitored by SDS-PAGE followed either by staining with Coomassie blue or by immunoblotting as described above.

DNA-binding assays. Electrophoretic mobility shift assays were performed as previously described, by using bacterial extracts and a radiolabelled *mim-1* A site oligonucleotide probe (9, 35). Free and bound DNAs were detected by autoradiography with Kodak XAR-5 film.

RESULTS

Construction and expression of hybrid *Drosophila* Myb–v-Myb retroviruses. The region of *Drosophila myb* encoding residues homologous to the DNA-binding domain of v-Myb was constructed by pairwise hybridization and ligation of overlapping synthetic oligonucleotides. This DNA fragment was then used to construct an avian retroviral vector designed to produce a protein in which the second and third repeats of *Drosophila* Myb had replaced the homologous region of v-Myb (Fig. 1). Following the introduction of a silent restriction enzyme site between the second and third *Drosophila* Myb repeats, related viruses were also constructed in which only the second or third *Drosophila* Myb repeat replaced the homologous repeat of v-Myb. To test for production of the proper proteins, the plasmid DNAs containing these proviruses were each transfected into quail QT6 cells. Two days later, the cells were harvested and Myb protein expression was assayed by immunoblotting (Fig. 2A). Similar levels of immunoreactive Myb proteins with a relative mobility of 45 kDa were detected in cells transfected with each of the proviral DNAs. These data demonstrated that each of the hybrid *Drosophila* Myb–v-Myb proviral DNAs produced a steady-state level of protein similar to that of the v-Myb control.

Transformation of hematopoietic cells by hybrid *Drosophila* Myb–v-Myb proteins. To test the ability of these hybrid Myb proteins to transform primary hematopoietic cells, the *neo-myb* proviral DNAs were each cotransfected into QT6 cells with MAV-1 helper viral DNA. Because v-Myb has no selectable phenotype in fibroblasts, the transfected cells were selected for *neo* expression with G418. Supernatants from these drug-resistant cells were then assayed for production of *neo-myb* viruses by infecting fresh QT6 cells. Similar numbers of G418-resistant colonies were scored for each virus (data not shown). The infected cells were then assayed for expression of Myb proteins by immunoblotting (Fig. 2B). In each case, similar levels of immunoreactive Myb proteins with a relative mobility of 45 kDa were detected in infected cells. These data demonstrated that all of the *neo-myb* viruses had similar titers

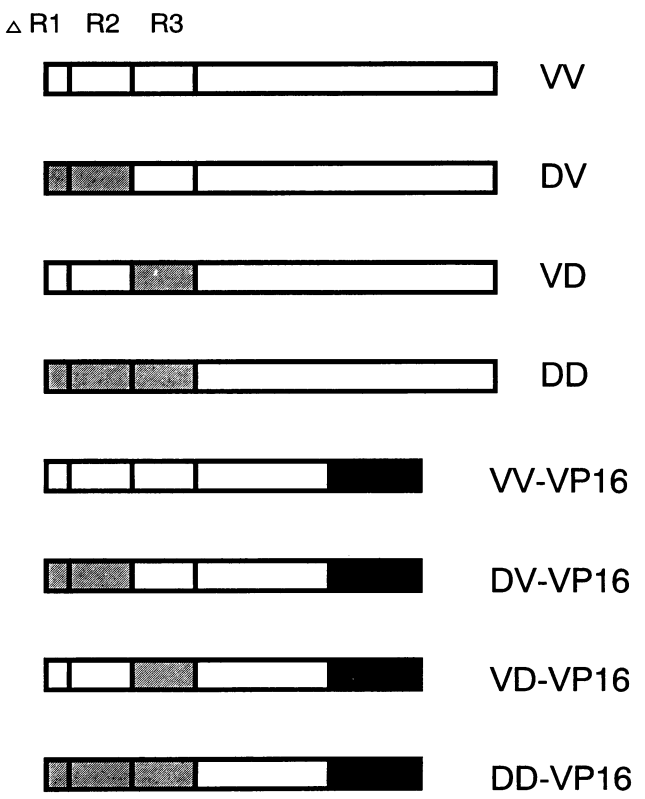

FIG. 1. Schematic diagrams of the various Myb proteins. The three small boxes at the left of each protein represent the Myb repeats (R1, R2, and R3), the first of which has been truncated in v-Myb (VV). Residues from v-Myb are unshaded, residues from *Drosophila* Myb are shaded, and residues from VP16 are solid black.

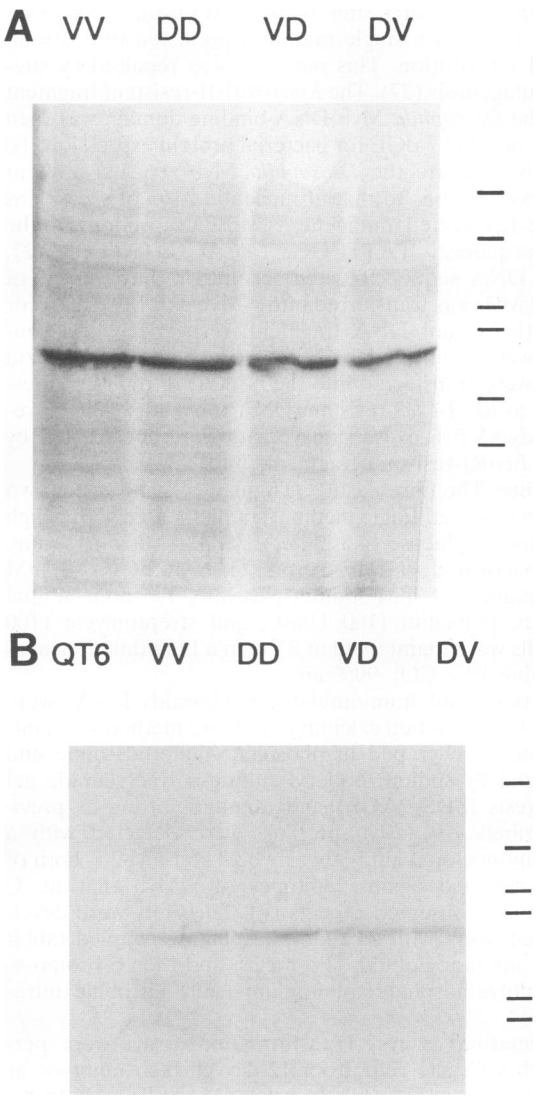

FIG. 2. Proviral expression of Myb proteins. (A) QT6 cells were transiently transfected with proviral DNAs encoding the indicated proteins. Two days later, the cells were harvested and lysates were analyzed by SDS-PAGE and immunoblotting with anti-Myb monoclonal antibodies. (B) QT6 cells, either uninfected (QT6) or infected with the indicated proviruses and selected for resistance to G418, were then analyzed for Myb protein expression as for panel A. The lines to the right of each panel represent molecular size standards (116, 84, 58, 48, 36, and 26 kDa, from top to bottom).

and produced similar steady-state levels of the predicted Myb proteins.

The transfected G418-resistant QT6 cells were also used in cocultivations to infect primary yolk sac cells isolated from 12-day chicken embryos. This tissue is particularly rich in immature myelomonocytic precursor cells. Uninfected control yolk sac cells differentiated into mature macrophages during the first 2 weeks of culture. However, infection with the virus encoding v-Myb caused proliferation of large numbers of small, round, highly refractile cells (Fig. 3A). Similar proliferation was observed with the viruses encoding hybrid proteins with either the second or third *Drosophila* Myb repeat. However, no proliferation was observed with the virus encoding the hybrid protein with both the second and third *Drosophila* Myb repeats.

The cells transformed by the hybrid proteins containing either the second or third *Drosophila* Myb repeat were indistinguishable from v-Myb-transformed monoblasts following cytocentrifugation and staining (Fig. 3A). Treatment of all three types of transformed cells with tetradecanoyl phorbol acetate (TPA) resulted in their differentiation into macrophage-like cells (Fig. 3B), suggesting that each of these Myb proteins transformed cells committed to the monocyte-macrophage lineage. This conclusion was further supported by fluorescence-activated cell sorter analysis with the Mo-1 monoclonal antibody, which detects an antigen expressed at high levels on the surface of v-Myb-transformed monoblasts (31). All three types of transformed yolk sac cells expressed similar levels of the Mo-1 antigen (Table 1). These levels were higher than that seen on the HD11 v-Myc-transformed macrophage-like cell line but lower than that seen on the BM2 v-Myb-transformed monoblastic cell line. Treatment of all three types of transformed cells with TPA caused a decrease in Mo-1 antigen expression similar to that observed with BM2 cells.

All three types of transformed yolk sac cells contained a Myb protein of the expected electrophoretic mobility when assayed by immunoblotting (Fig. 4). To be certain that the cells contained the predicted hybrid viruses rather than possible contamination with wild-type stocks, the genomic DNA from these cells was analyzed by polymerase chain reaction. The second *Drosophila* Myb repeat contains an *Apa*I restriction site not present in v-Myb, whereas the third repeat of v-Myb

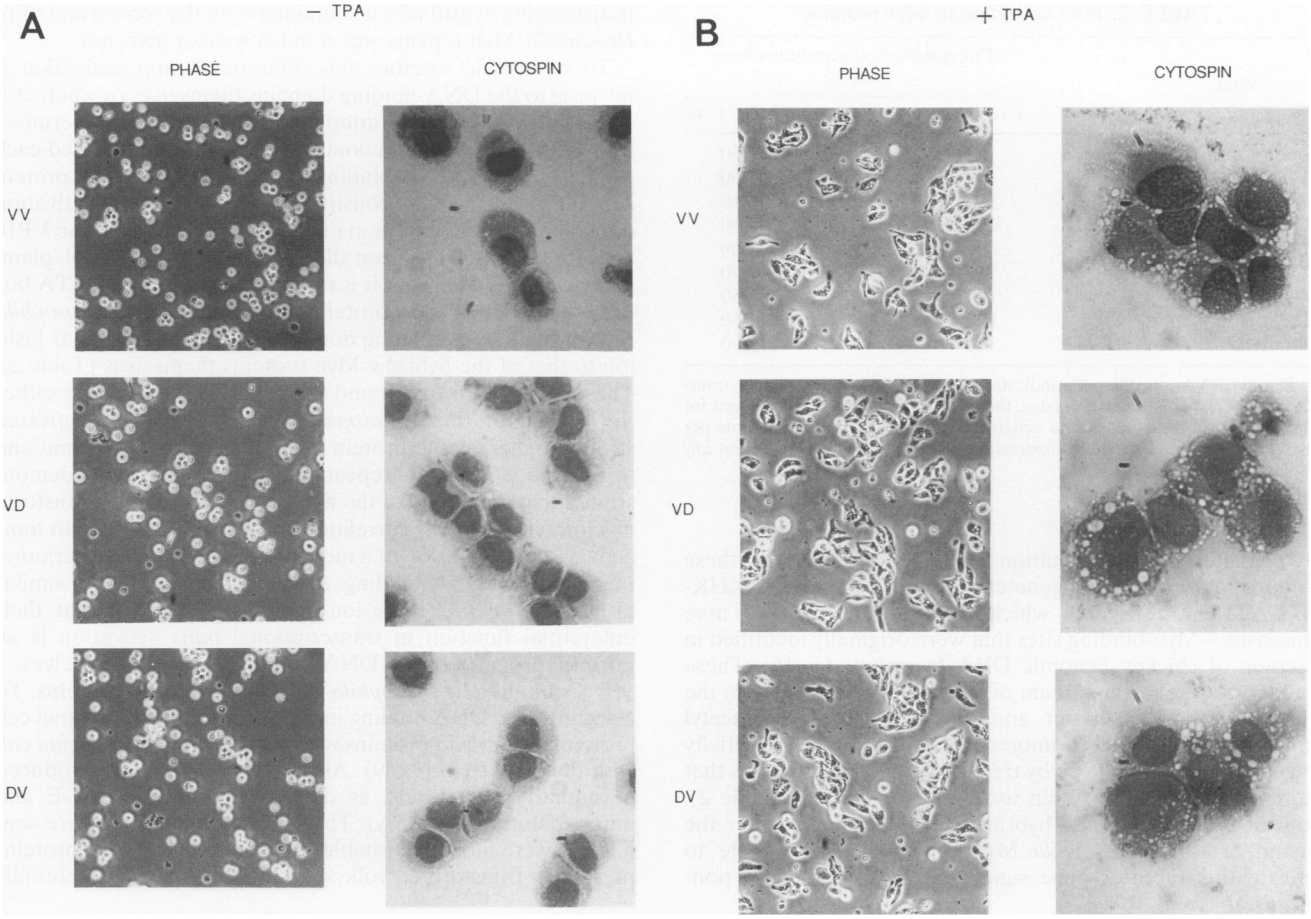

FIG. 3. Transformation of primary hematopoietic cells. (A) Chicken embryonic yolk sac cells transformed by the indicated viruses were visualized by phase contrast microscopy (PHASE) or by cytocentrifugation and staining (CYTOSPIN). (B) Transformed cells from the same cultures were treated with TPA (250 ng/ml) daily for 4 days and then visualized as for panel A.

contains an *Ear*I restriction site not present in the third repeat of *Drosophila* Myb. These restriction enzymes were therefore used to verify the structures of the integrated proviruses in the DNA amplified from the transformed cells (data not shown).

Transcriptional *trans* activation by hybrid *Drosophila* Myb–v-Myb proteins. Because substitution of one, but not both, of the v-Myb repeats with the homologous repeats of *Drosophila*

TABLE 1. Mo-1 antigen expression on transformed cells[a]

Virus	Relative fluorescence intensity	
	Untreated	TPA treated
VV	250	120
DV	240	150
VD	250	140
BM-2 (v-Myb)	480	180
HD-11 (v-Myc)	113	ND

[a] Yolk sac cells transformed by the indicated viruses were incubated with monoclonal antibody Mo-1 and then fluoresceinated goat anti-rabbit immunoglobulin. Relative fluorescence intensity was determined with a fluorescence-activated cell sorter (Becton Dickinson). For comparison, an established v-*myb*-transformed monoblast cell line (BM2) and an established v-*myc*-transformed macrophage-like cell line (HD11) were also analyzed. Some cells were treated with TPA (250 ng/ml) daily for 4 days prior to analysis. ND, not done.

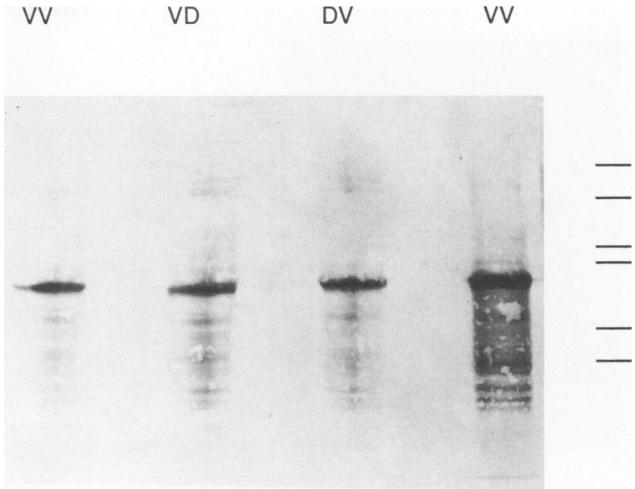

FIG. 4. Myb protein expression in transformed hematopoietic cells. Lysates of the cells in Fig. 3A were analyzed by SDS-PAGE and immunoblotting with anti-Myb monoclonal antibodies. In addition, v-Myb protein produced in bacteria (see Fig. 6) was loaded in the far right lane. The lines to the right represent molecular size standards, as described in the legend to Fig. 2.

TABLE 2. *trans* activation by Myb proteins[a]

Virus	Chloramphenicol acetyltransferase activity	
	E1B-CAT	KHK-E1B-CAT
None (vector only)	90	150
VV (v-Myb)	100	5,060
DV	100	3,210
VD	110	3,700
DD	80	760
VV-VP16	110	20,090
DV-VP16	100	13,150
VD-VP16	120	11,160
DD-VP16	90	1,190

[a] Plasmid DNAs encoding the indicated activators and reporters were transfected into QT6 cells. Two days later, the cells were harvested and assayed for chloramphenicol acetyltransferase activity. The numbers represent counts per minute above the background of extraction of a reaction mixture without any cellular lysate.

Myb resulted in transformation, we tested the ability of these proteins to *trans* activate gene expression. We used the KHK-EB-CAT reporter gene, which contains an array of nine consensus v-Myb-binding sites that were originally identified in a screen of chicken genomic DNA fragments (1, 16). These binding sites lie just upstream of a simple TATA box from the adenovirus E1B promoter and the chloramphenicol acetyl transferase gene. This promoter has a very low basal activity and can be readily induced by transiently transfected DNA that expresses the v-Myb protein used in these studies (Table 2). Both of the transforming hybrids which contained either the second or third *Drosophila* Myb repeat were also able to activate this reporter gene significantly. However, the non-transforming hybrid which contained both the second and third *Drosophila* Myb repeats was a much weaker activator.

To determine whether this differential *trans* activation is intrinsic to the DNA-binding domains themselves or whether it represents a differential interaction with the more C-terminal transcriptional activation domain of v-Myb, we expressed each of these hybrid DNA-binding domains as a fusion protein containing the strong constitutive transcriptional activation domain of the VP16 protein of herpes simplex virus. The VP16 activation domain has been shown to function in animal, plant, and yeast cells whenever it is tethered adjacent to a TATA box (39). The VP16 fusion proteins containing hybrid *Drosophila* Myb–v-Myb DNA-binding domains behaved in a similar fashion to that of the hybrid v-Myb proteins themselves (Table 2). The v-Myb–VP16 fusion and those proteins containing either the second or third *Drosophila* Myb repeat *trans* activated strongly, whereas the protein containing both the second and third *Drosophila* Myb repeats did not. These data demonstrated that the ability of the hybrid Myb proteins to transform myelomonocytic cells correlated well with their ability to *trans* activate the expression of a model reporter gene. Furthermore, these hybrid DNA-binding domains behaved in a similar fashion in Myb-VP16 fusion proteins, suggesting that their differential function in transcriptional *trans* activation is an intrinsic property of the DNA-binding domains themselves.

DNA binding by *Drosophila* Myb–v-Myb hybrid proteins. To assay intrinsic DNA binding in the absence of other animal cell proteins, the hybrid proteins were expressed in *Escherichia coli* with the T7 system (9, 49). All of the proteins were produced at similarly high levels, as determined by SDS-PAGE and immunoblotting (Fig. 5A). Their relative mobilities were similar and were indistinguishable from those of the Myb proteins present in transformed yolk sac cells (Fig. 4). The bacterially

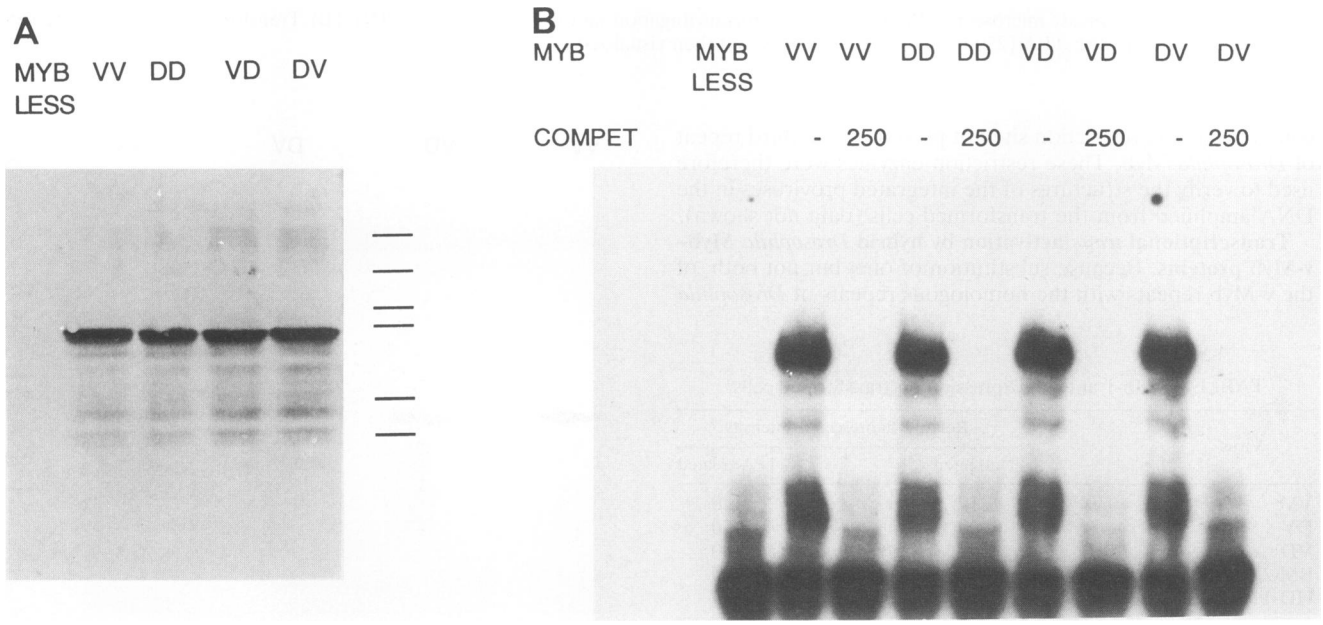

FIG. 5. DNA binding by Myb proteins produced in bacteria. (A) Plasmids containing the indicated reading frames under control of T7 RNA polymerase were induced for expression. A plasmid without a Myb open reading frame was used as a control (MYBLESS). Equal amounts of total protein were analyzed by SDS-PAGE and immunoblotting with anti-Myb monoclonal antibodies. The lines to the right represent molecular size standards, as described in the legend to Fig. 2. (B) The same extracts shown in panel A were incubated with a radiolabelled oligonucleotide containing the *mim-1* A Myb-binding site in the presence (250) or absence (−) of a 250-fold excess of the same unlabelled oligonucleotide as a competitor (COMPET). Complexes were detected by electrophoresis in nondenaturing gels and autoradiography.

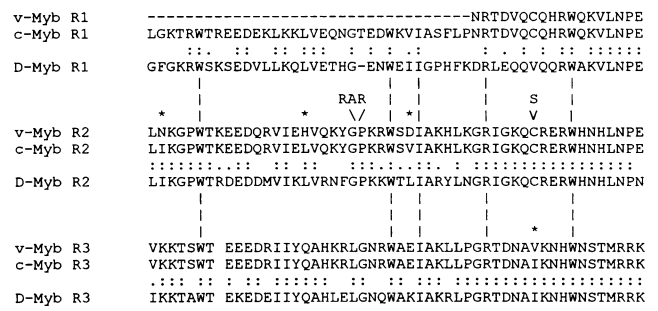

FIG. 6. Comparison of the v-Myb and *Drosophila* Myb DNA-binding domains. The amino acid sequences of the avian myeloblastosis virus v-Myb, chicken c-Myb, and *Drosophila* Myb (D-Myb) DNA-binding domains were aligned. Amino acid identities (:) and similarities (.) are indicated. Residues conserved in all three repeats (R1, R2, and R3) are indicated by vertical bars. Amino acid substitutions in v-Myb relative to c-Myb are also indicated (*). The positions of linker insertion mutation 281 (RAR) and C65S (S) are indicated.

expressed proteins were subjected to electrophoretic mobility shift assays using the strong *mim-1* A type Myb-binding site as a probe (Fig. 5B). All three hybrid *Drosophila* Myb–v-Myb proteins bound to this DNA in a fashion similar to that of v-Myb itself. In each case, binding was specifically inhibited by an excess of the unlabelled probe used as a competitor. In addition, the hybrid containing both the second and third repeats of *Drosophila* Myb was shown to bind to a series of 12 mutant Myb sites in a manner similar to that of v-Myb itself and to that of a similar hybrid containing the *Dictyostelium* Myb DNA-binding domain (data not shown) (9, 47). These data demonstrate that although the hybrid containing both the second and third repeats of *Drosophila* Myb does not transform or *trans* activate well, it appears to bind to DNA as well as wild-type v-Myb and the transforming variants containing only the second or third *Drosophila* Myb repeat.

DISCUSSION

The v-Myb protein appears to transform cells of the myelomonocytic lineage by binding directly to DNA and regulating gene expression. Analysis of a series of v-Myb mutants had identified two mutations in the second repeat of the DNA-binding domain that interfere with transcriptional activation and transformation but not with DNA binding itself (Fig. 6) (16, 28). Because the Myb DNA-binding domain has been highly conserved during metazoan evolution, we used the homologous region of *Drosophila* Myb as a naturally occurring mutant with which to investigate this phenomenon further. Our results demonstrated that substitution of either the second or third *Drosophila* Myb repeat into v-Myb was compatible with transformation of myelomonocytic cells. However, substitution of both of the *Drosophila* Myb repeats into v-Myb resulted in a protein that was incapable of transformation. These hybrid proteins showed a strong correlation between transformation and the ability to transcriptionally activate a model reporter gene. This correlation was maintained with fusion proteins containing the hybrid DNA-binding domains and the strong transcriptional activation domain of VP16. These results imply that the differences in transformation and transcriptional activation are due to functional differences in the DNA-binding domains themselves. However, when the same proteins were expressed in *E. coli*, no differences in DNA binding were observed. We therefore hypothesize that the highly conserved Myb repeats also function in specific protein-protein interactions. This model is consistent with previous results which demonstrated that although the amino acid substitutions in v-Myb are not required for transformation, reversion of single substitutions within the DNA-binding domain could alter the phenotype of transformed cells (21, 48). In addition, our results obtained with *Drosophila* Myb–v-Myb hybrids suggest that both the second and third repeats of v-Myb determine specific protein-protein contacts because the presence of either v-Myb repeat is sufficient for transformation and transcriptional activation. In this regard, we have recently shown that the binding of bacterially expressed v-Myb to immobilized nuclear proteins requires the highly conserved Myb repeats (46).

Other Myb proteins have been shown to interact with specific partner proteins to stimulate transcription. For example, the Myb-related BAS1 protein of *S. cerevisiae* requires the presence of the BAS2/PHO2 homeodomain protein to activate the *HIS4* promoter strongly (50). In addition, the Myb-related C1 protein of *Zea mays* requires the presence of either the B or R helix-loop-helix protein to activate the *Bz1* promoter strongly (12, 41). Furthermore, it has recently been shown that both the Gag-Myb-Ets protein encoded by the E26 virus and c-Myb require the C/EBP-related NF-M protein to activate the *mim-1* promoter strongly (34). In all of these cases, the partner proteins are bound to adjacent sites within the promoter and no conclusive evidence for direct protein-protein interactions has been provided. In our studies, we used a simple reporter gene which appears to consist of only Myb-binding sites and a TATA box. Therefore, we believe that the mutations that affect transcriptional activation but not DNA binding in vitro are likely to alter the binding of adapter proteins that permit v-Myb to interact with the basal transcriptional machinery. These mutations could either directly alter binding to the adapter, or prevent a posttranslational modification of v-Myb that is essential for this binding. An alternative explanation is that efficient DNA binding in vivo requires an additional protein that is not essential for our in vitro DNA-binding assays. Such a facilitator protein has recently been described for a mammalian homeodomain protein (32). However, if such a protein exists for v-Myb it must be quite small or act transiently, because protein-DNA complexes formed by nuclear extracts containing v-Myb appear to comigrate with bacterially expressed v-Myb in nondenaturing gels (6, 24).

The results presented here should provide a useful framework for elucidating the precise amino acids required for the complex role of the Myb repeats in transcriptional activation and leukemic transformation. The partial first repeat and complete second repeat of v-Myb are 68% identical to the homologous region of *Drosophila* Myb (Fig. 6). The third repeat of v-Myb is 80% identical to the third repeat of *Drosophila* Myb. Nevertheless, these small numbers of evolutionarily selected amino acid substitutions which do not affect DNA binding are sufficient for differential function in animal cells.

ACKNOWLEDGMENTS

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