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Transcription regulation by murine B-*myb* is distinct from that by c-*myb*

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ABSTRACT

The transcription regulatory properties of murine B-*myb* protein were compared to those of c-*myb*. Whereas c-Myb trans-activated an SV40 early promoter containing multiple copies of an upstream c-Myb DNA-binding site (MBS-1), and similarly the human c-*myc* promoter, B-Myb was unable to do so. Full-length B-Myb translated *in vitro* did not bind MBS-1; however, truncation of the B-Myb C-terminus or fusion of the B-Myb DNA-binding domain to the c-Myb C-terminus showed that it was inherently competent to interact with this motif. Further evidence from co-transfection experiments, demonstrating that B-Myb inhibited trans-activation by c-Myb, suggested that failure of B-Myb to trans-activate these promoters did not simply occur through lack of binding to MBS-1. Moreover, using GAL4/B-Myb fusions, it was found that an acidic region of B-Myb, which by comparison to c-Myb was expected to contain a transcription activation domain, actually had no inherent trans-activation activity and indeed appeared to trans-inhibit c-Myb. In contrast to the above findings, both B-Myb and c-Myb were able to weakly trans-activate the DNA polymerase alpha promoter. Results obtained here demonstrate that the activities of B-Myb and c-Myb are clearly distinct and suggest that these related proteins may have different functions in regulation of target gene expression.

INTRODUCTION

B-*myb* is a member of a family that includes the proto-oncogene c-*myb*, a gene essential for the development of the haemopoietic system (1), and A-*myb*. B-*myb* cDNA's have been isolated from both human and mouse cells (2,3) and their predicted protein products show especially marked homology to the extensive DNA-binding domain of c-Myb. It therefore seems likely that B-Myb functions as a sequence-specific DNA-binding protein and transcription regulator as does the prototypic c-Myb protein (4–7). In support of this notion, human B-Myb binds DNA with very similar, though not identical, sequence specificity as c-Myb (8,9). Moreover, human B-Myb was reported to trans-activate an SV40 promoter containing multiple copies of the c-Myb-binding site MBS-1 (8) and also the human c-*myc* promoter (10).

Despite apparent functional similarities, c-Myb and B-Myb show limited homology outside the DNA-binding domain; only in a segment of B-Myb located towards the C-terminus is there further significant conservation of sequence with c-Myb (2,3). It has been suggested that the c-Myb C-terminus constitutes a negative regulatory domain, controlling DNA-binding and trans-activation activities (6,11). Sequences involved in inhibitory effects on trans-activation extend upstream of the region conserved with B-Myb, however, encompassing a potential leucine zipper structure that has no counterpart in B-Myb (12). It is notable that the acidic c-Myb trans-activation domain, which is located between the DNA-binding and negative regulatory domains near the centre of the primary amino acid sequence, shows no homology with the equivalent region of B-Myb. This suggests that B-Myb contains an unrelated transcription regulatory domain. Indeed, we noted (3) that this region of B-Myb is strongly conserved between the human and murine homologues and moreover is of somewhat acidic character, typical of many trans-activation domains. We report here that, contrary to expectation, mouse B-Myb was unable to trans-activate either an SV40 early promoter containing multiple Myb-binding sites or the human c-*myc* promoter. Furthermore, B-Myb was found to antagonise trans-activation of these promoters by c-Myb.

MATERIALS AND METHODS

Plasmids

The reporter plasmid pMBS-SVcat was constructed by first inserting a *Sph*I/*Bam*HI fragment from pSV2cat (containing the SV40 early promoter linked to the chloramphenicol acetyltransferase gene) into equivalent sites of pUC18 (yielding pSVcat). Eight tandemly orientated copies of an MBS-1 oligonucleotide (AGAATGTGTGTCAGTTAGGGTGTAGAG) were then inserted into the *Hin*dIII site derived from pUC18. The human DNA polymerase alpha promoter reporter plasmid, pDPαcat, was made by inserting a *Nde*I/*Ava*II fragment corresponding to nucleotides −542/+45 of this promoter (13) into *Nde*I/*Sal*I sites of pUCcat. This deleted a cluster of Myb-binding sites that are fortuitously present in the pUC18 *Nde*I/*Hin*dIII fragment. The human c-*myc* promoter reporter, pHmycCAT, contains the 2.5kb *Hin*dIII/*Nae*I fragment that encompasses the P1 and P2 promoters (14) linked to the CAT gene.

B-*myb* plasmids were constructed from the mouse pMB21 cDNA (3); pSVB-myb contains the full-length coding sequence from a *Xho*I site in the 5′ non-coding region to a vector-derived

*Hin*dIII site downstream of B-*myb* sequences cloned in the SV40 early promoter expression vector pKC4 (obtained from D.Hanahan). The c-*myb* expression vector pSVC-myb encodes authentic mouse c-Myb from a cDNA sequence derived from pJ4myb (15); this was recloned in pKC4. *In vitro* mutagenesis of pSVC-myb to introduce a *Sal*I site at codons 193–194, thus generating pSVC-mybSal was done by inserting an oligonucleotide containing these mutations (GTGGAA to GTCGAC) between flanking *Eco*RI and *Pst*I sites. Fusions with GAL4 were made in the vector pMG147Spe, which encodes the first 147 amino acids (aa) of GAL4 under the control of the MLV promoter/enhancer. B-*myb*, c-*myb* and HSV-1 VP16 fragments encoding the amino acids indicated in the text were fused in-frame downstream of GAL4 at a filled-in *Sal*I site or between *Sal*I and an adjacent filled-in *Spe*I site. Translation of fusion proteins would in each case be expected to terminate at a nonsense codon contained within the *Spe*I site or at the natural C-termini of B-Myb or VP16. The pUAScat reporter contained five copies of a GAL4 binding site (CTCCGCTCGGAGGACAGTA) cloned upstream of the HSV-1 thymidine kinase promoter in the vector pBLcat2 (16).

Reporter gene assays

Plasmids were introduced into cultures of NIH3T3 cells (approximately 50% confluent) by calcium phosphate precipitation (17). Typically, 4μg of the reporter plasmid was co-precipitated with 4μg of the effector plasmid and 2μg of pJ4Ωβ-gal, encoding β-galactosidase activity under control of the Moloney MuLV LTR promoter/enhancer. Controls which lacked effector, or where the amount was reduced, contained a quantity of the empty vector (i.e. pKC4 or pMG147Spe) to bring the total to 10μg. Cell lysates were made 2 days after transfection and normalised by assay of β-galactosidase activity using chlorophenolred β-D-galactopyranoside (Boehringer Mannheim) as a substrate. CAT activity was measured as described previously (18). Reporter gene assays were performed at least twice and were in most instances repeated many times, especially (as illustrated in Figure 1) where some variation in the level of trans-activation was observed.

DNA-binding assays

B-*myb* proteins were translated *in vitro* after insertion of a full-length cDNA coding sequence into the vector pT7βΔ6Sal (19), which results in efficient translation in reticulocyte lysates presumably by virtue of the 5′ untranslated β-globin leader. Translation of c-*myb* proteins using this vector has been described previously (19). The B-*myb* and c-*myb* plasmids (1μg) were transcribed with T7 RNA polymerase and translated in a coupled reticulocyte lysate reaction (Promega); translation of full-length proteins was achieved with supercoiled plasmid and that of C-terminally truncated proteins by prior cleavage of plasmids with *Sca*I, *Aat*II or *Pvu*II (for B-*myb*) and *Sma*I (for c-*myb*). Parallel reactions were set up in which proteins were labelled by the addition of ^{35}S-cysteine; these proteins were analysed by autoradiography following resolution on a 10% SDS-PAGE gel. Bandshift assays were performed essentially as described previously (19), using a double-stranded MBS-1 binding site oligonucleotide (AGAATGTGTGTCAGTTAGGGTGTAGAG) ^{32}P-labelled with γ-ATP and polynucleotide kinase. Reactions contained 5μl of the programmed reticulocyte lysate, 0.5ng of the labelled oligonucleotide and 1μg poly(dI/dC) and 1μg denatured calf thymus DNA as non-specific competitors. Where appropriate, 100ng of the unlabelled MBS-1 oligonucleotide was added to reactions as a specific competitor.

RESULTS

Trans-activation properties of mouse B-*myb*

DNA sequences comprising the full-length coding regions of B-*myb* and c-*myb* were placed under transcriptional control of the SV40 early promoter in the pKC4 expression vector. These plasmids (pSVB-myb and pSVC-myb, respectively) were transfected into NIH3T3 cells together with a CAT reporter plasmid (pMBS-SVcat) containing an enhancerless SV40 early promoter, upstream of which were inserted multiple copies of the MBS-1 Myb-binding site (5). As expected from previous work (5), significant trans-activation of the reporter (an average of five experiments gave a value of 7.3-fold) was obtained by co-transfection with 4μg pSVC-myb (Figure 1, column 6). It was observed in control experiments (not shown here) that pSVcat, which lacks the multiple MBS-1 sites, was not subject to trans-activation by pSVC-myb, indicating a requirement for specific DNA-binding by c-Myb for trans-activation of this promoter; similar results have been obtained elsewhere (5). Surprisingly, trans-activation of pMBS-SVcat was not observed upon co-transfection with each of 1μg, 2μg, 4μg or 10μg of pSVB-myb (Figure 1, columns 2–5). This result was unexpected as human B-Myb has been reported to trans-activate a similar MBS-1 binding site/SV40 early promoter in CV-1 cells (8).

The activity of c-Myb and B-Myb was also tested on the human DNA polymerase alpha (DPα) promoter in a similar co-transfection assay. Previously, it had been reported that DPα expression is dependent on c-Myb function in T cells (20), suggesting the possibility that Myb proteins may directly act on DPα transcription; however, the sequence of the DPα promoter revealed no consensus Myb-binding sites (13). It was found here that the DPα promoter was nonetheless subject to on average 3.4-fold trans-activation by c-Myb (Figure 1); additional experiments (not shown here) indicate that this effect, unlike that with the pMBS-SVcat reporter, is dependent neither on specific DNA-binding nor on the recognised trans-activation domain of c-Myb (7) and may be akin to the activity of v-Myb on the HSP70 promoter (21). It was apparent that B-Myb was similarly able to trans-activate the DPα promoter (Figure 1, column 11). This result indicates that the failure of B-Myb to trans-activate pMBS-SVcat was not due simply to a deficiency in induction of B-Myb protein in transfected cells. This conclusion is strengthened by the finding that maximal induction of pDPαcat was obtained with 4μg pSVB-myb and that some 'squelching' with a greater amount of plasmid was apparent (Figure 1, columns 8–10).

Binding of mouse B-Myb to MBS-1

To determine whether failure of mouse B-Myb to trans-activate the pMBS-SVcat reporter was due to an inability to recognise the MBS-1 DNA-binding motif, band-shift assays were performed with a ^{32}P-labelled oligonucleotide comprising this sequence. To generate sufficient protein for this analysis, DNA sequences encoding full-length mouse B-Myb were inserted downstream of a T7 promoter and a β-globin 5′ non-translated sequence, generating plasmid pT7β-B-myb, so that they could be efficiently transcribed and translated *in vitro* by a coupled reaction in a rabbit reticulocyte lysate. The transcription/translation reaction was

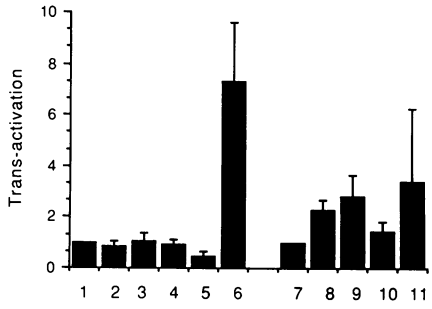

Figure 1. Trans-activation by B-Myb and c-Myb. CAT activity was assayed in extracts of NIH3T3 cells transfected with pMBS-SVcat and pDPαcat reporters together with the control plasmid pKC4 or the effectors pSVB-myb or PSVC-myb. Trans-activation in each of five experiments was calculated assuming a value of unity for control pKC4 co-transfections, and the average is represented by the bar graph. Standard deviations are shown above each column. Co-transfections contained 4μg pMBS-SVcat (columns 1−6) or 4μg pDPαcat (columns 7−11) together with 4μg pKC4 (columns 1 & 7), 1μg pSVB-myb (columns 2), 2μg pSVB-myb (columns 3 & 8), 4μg pSVB-myb (columns 4 & 9), 10μg pSVB-myb (columns 5 & 10) and 4μg pSVC-myb (columns 6 & 11).

programmed either with supercoiled pT7β-B-myb or with pT7β-B-myb linearised by digestion with *Sca*I, *Aat*II or *Pvu*II; these enzymes cut within the B-*myb* coding sequence and the resultant RNA's would be expected to encode proteins C-terminally truncated at aa 508, 349 and 226, respectively. A similar plasmid, pT7βmyb, was used to generate *in vitro*-translated c-Myb.

Analysis of ^{35}S-cysteine-labelled *in vitro* translated proteins by SDS-PAGE (Figure 2A) indicated that full-length B-Myb (track 1) had an apparent size estimated by gel mobility of approximately 105kDa, which is notably greater than its predicted molecular weight of 79kDa (3). In comparison the molecular weight of c-Myb was estimated here to be 80kDa, which is rather closer to its predicted size of 70kDa. Truncation of B-Myb resulted in proteins that also had anomalously high estimated molecular weights; for example the coding sequence truncated at the *Sca*I site (encoding aa 1−508), predicted to encode a 57kDa protein, actually had an apparent molecular weight of 75kDa (Figure 2A, track 2). These discrepancies suggest that B-Myb may adopt an unusual conformation or possibly be modified post-translationally in the reticulocyte lysate. Some support for the latter possibility was given by the observation that two or even three forms of the protein with slightly different mobility were apparent with full-length and B-*myb* proteins truncated at aa 508 and 349 (Figure 2A, tracks 1−3). In contrast, a single band was evident with the B-Myb protein truncated at aa 226 (track 4).

Bandshift assays were performed with batches of unlabelled B-Myb and c-Myb proteins translated *in vitro* in parallel reactions (Figure 2B). It was found that full-length B-Myb did not bind to DNA, although the three truncated proteins did so to varying degrees. Thus, a weak bandshift was obtained with B-Myb truncated at aa 508 (Track 3) and progressively stronger bandshifts were obtained with proteins truncated at aa 349 and aa 226 (Tracks 5 & 7). In comparison, both full-length and truncated c-Myb bound strongly to the MBS-1 oligonucleotide (Figure 2B, tracks 9 &11). These findings indicate that the B-Myb DNA-binding domain is able to recognise the MBS-1 site. They further suggest, however, that C-terminal sequences of B-Myb may affect the ability of the protein to bind DNA,

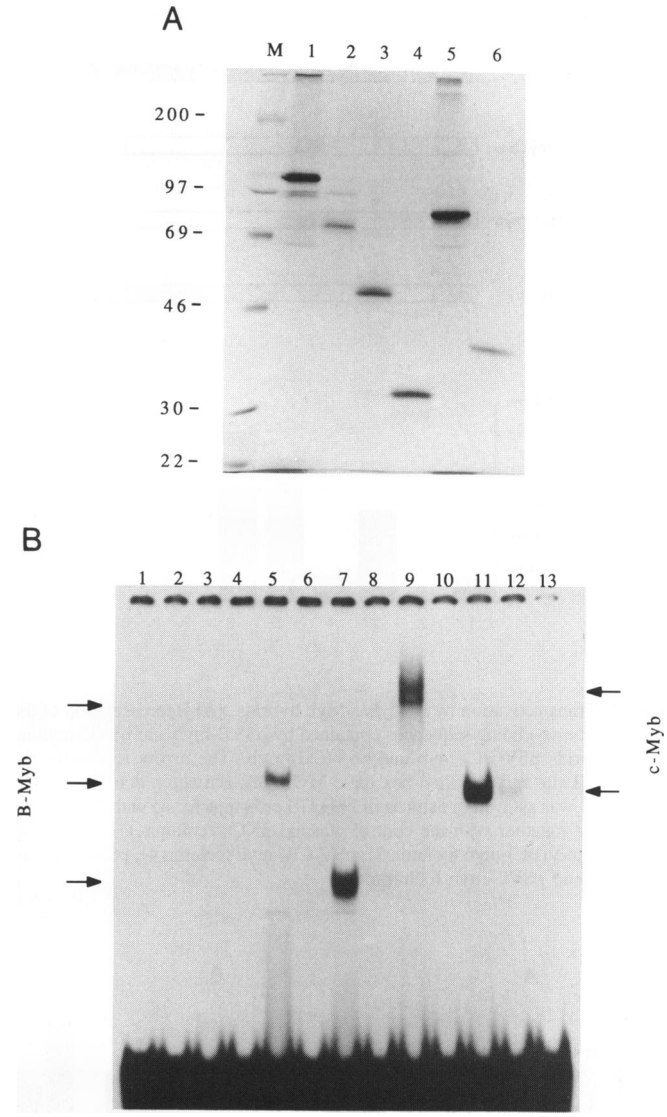

Figure 2. Sequence-specific DNA-binding by B-Myb. **(A)** B-Myb and c-Myb proteins were translated *in vitro* using a coupled transcription/translation sytem in rabbit reticulocyte lysates primed with the plasmids pT7β-B-myb and pT7βmyb. Synthesis of C-terminally truncated proteins was achieved by restriction of these plasmids as detailed in Materials and Methods. Proteins were labelled by incorporation of ^{35}S-cysteine and displayed by SDS-PAGE and autoradiography and comprised full-length B-Myb (track 1), B-Myb aa 1−508 (track 2), B-Myb aa 1−349 (track 3), B-Myb aa 1−226 (track 4), full-length c-Myb (track 5) and c-Myb aa 1−297 (track 6). Molecular weight markers were run in an adjacent track (M) as indicated. **(B)** Complexes formed between unlabelled *in vitro* translated B-Myb and C-Myb proteins and 0.5ng of a ^{32}P-labelled MBS-1 oligonucleotide were displayed by polyacrylamide gel electrophoresis and autoradiography. Binding reactions contained full-length B-Myb (tracks 1 & 2), B-Myb aa 1−508 (tracks 3 & 4), B-Myb aa 1−349 (tracks 5 & 6), B-Myb aa 1−226 (tracks 7 & 8), full-length c-Myb (tracks 9 & 10), c-Myb aa 1−297 (tracks 11 & 12) and control reticulocyte lysate (track 13). One of each duplicate binding reaction (tracks 2,4,6,8,10 & 12) also contained 100ng of unlabelled MBS-1 oligonucleotide as a competitor. The positions of the complexes formed with B-Myb and c-Myb are indicated.

presumably reflecting post-translational modification of the protein that can occur in the reticulocyte lysate.

To determine whether the B-Myb DNA-binding domain could recognise the MBS-1 site *in vivo*, hybrids between B-Myb and

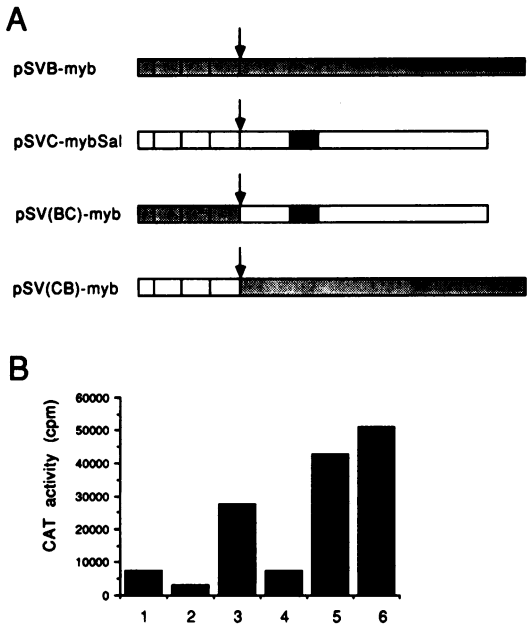

Figure 3. Trans-activation by B-Myb/c-Myb hybrids. **(A)** Representation of the B-*myb* and c-*myb* coding sequences contained by pSVB-myb and pSVC-mybSal and the hybrids pSV(BC)-myb and pSV(CB)-myb. The arrow represents the common *SalI* site and the filled-box the c-Myb trans-activation domain (7). **(B)** CAT activity was assayed in extracts of NIH3T3 cells transfected with the reporter pMBS-SVcat together with the control plasmid pKC4 (column 1), pSVB-myb (column 2), pSV(BC)-myb (column 3), pSV(CB)-myb (column 4), pSVC-mybSal (column 5) and pSVC-myb (column 6).

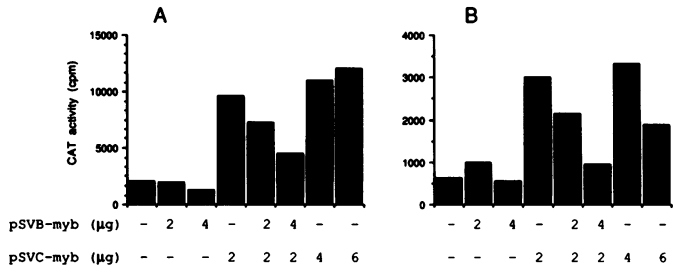

Figure 4. Inhibition of c-Myb trans-activation activity by B-Myb. **(A)** CAT activity was assayed in NIH3T3 cells transfected with 4μg pMBS-SVcat together with the indicated amounts of pSVB-myb and pSVC-myb. **(B)** CAT activity was assayed in NIH3T3 cells transfected with 4μg pHmycCAT together with the indicated effector plasmids.

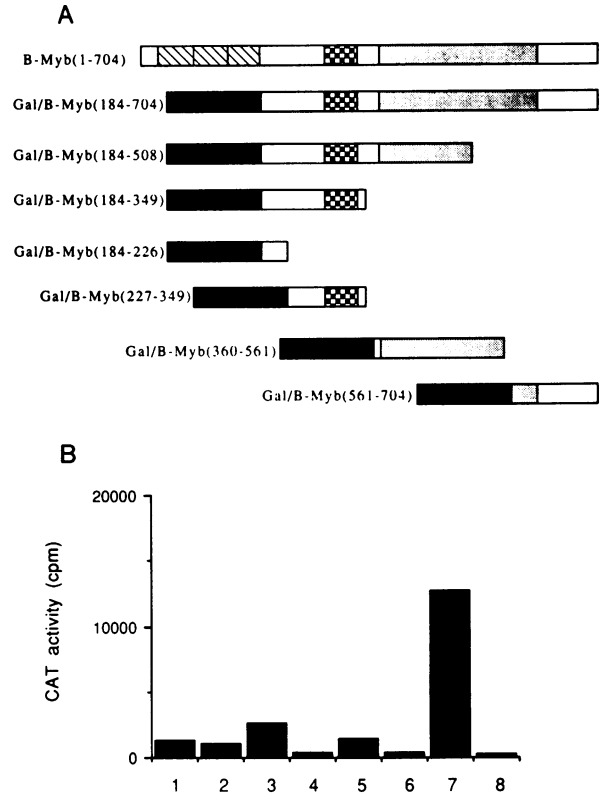

Figure 5. Analysis of possible B-Myb trans-activation domains by fusion with GAL4. **(A)** Representation of the structures of GAL4 and B-Myb fusion proteins. Regions of B-Myb highly conserved between the human and mouse homologues (3) are boxed; the hatched box represents the DNA-binding domain (aa 29–183), the chequered an acidic sequence (aa 284–335) and the stippled (aa 369–614) a sequence which contains some homology with c-Myb and A-Myb (2,3). GAL4 sequences (aa 1–147) fused to B/Myb are represented by the filled box and include the DNA-binding and dimerisation domains. **(B)** CAT activity was assayed in NIH3T3 cells transfected with 4μg pUAScat together with 4μg of the empty vector pMG147Spe (column 1) or the effector plasmids pGal/B-Myb(184–704) (column 2), pGal/B-Myb(184–508) (column 3), pGal/B-Myb(184–349) (column 4), pGal/B-Myb(184–226) (column 5), pGal/B-Myb(227–349) (column 6), pGal/B-Myb(360–561) (column 7) and pGal/B-Myb(561–704) (column 8). CAT activity obtained in a parallel transfection with the c-Myb trans-activation domain plasmid pGal/c-Myb(200–325) was 209,000 cpm.

c-Myb were constructed and tested for their activity on the pMBS-SVcat reporter. To facilitate this, a *SalI* site was introduced into the c-*myb* sequences of pSVC-myb at a site which encompasses the two codons immediately downstream from those encoding the DNA-binding domain. Such *in vitro* mutagenesis resulted in a single conservative amino acid change (Glu to Asp) at position 194 of c-Myb. Mouse B-*myb* contains a naturally occurring *SalI* site at the equivalent position. By joining B-*myb* and c-*myb* cDNA fragments at this common restriction site, hybrids consisting of the amino terminal c-Myb DNA-binding domain linked to B-Myb C-terminal sequences, and *vice versa*, were specified (Figure 3A). By co-transfection experiments it was found that the hybrid consisting of the B-Myb DNA-binding domain and c-Myb C-terminal sequences was able to trans-activate the pMBS-SVcat promoter (Figure 3B, column 3), while in contrast the hybrid containing the c-Myb DNA-binding and B-Myb C-terminal sequences was inactive (Figure 3B, column 4). These results indicated that the B-Myb DNA-binding domain was able to recognise the MBS-1 sites *in vivo*; however, they do not exclude the possibility that the C-terminus of B-Myb modifies this interaction.

Affect of B-*myb* on trans-activation by c-*myb*

As mouse B-Myb was consistently unable to trans-activate transcription of pMBS-SVcat, experiments were performed to address the question whether it may instead affect the ability of c-Myb to act on this promoter. Trans-activation of pMBS-SVcat by pSVC-myb was indeed found to be impaired in a dose-dependent fashion in co-transfections which included pSVB-myb (Figure 4A). Similarly, trans-activation of the human c-*myc*

promoter by c-Myb was also impaired in co-transfections containing pSVB-myb (Figure 4B). It is notable that mouse B-Myb itself had little or no trans-activation activity on the human c-*myc* promoter, as it has been reported that human B-Myb is a potent activator (10). The ability of B-Myb to inhibit trans-activation of these promoters by c-Myb is consistent with the notion, but does not prove, that it acts by competing for binding sites, suggesting that B-Myb does interact with MBS-1 *in vivo*.

Fusion of B-*myb* with GAL4

By analogy to c-Myb (6), the B-Myb C-terminus may potentially contain a negative regulatory domain that could modulate the ability of this protein to trans-activate. Moreover, from data obtained here it appears that the presence of the B-Myb C-terminus may, at least under certain conditions, inhibit DNA binding (Figure 2B) which could also affect this activity. To investigate the influence of the B-Myb C-terminus on trans-activation and to more critically define any regions of the protein that may constitute a transcription regulatory domain, fusions of various B-Myb sequences C-terminal of the DNA-binding domain were made with a sequence encoding the yeast GAL4 DNA binding domain (Figure 5A). These plasmids were co-transfected into NIH3T3 cells with a reporter construct (pUAScat) that contains an HSV-1 thymidine kinase promoter upstream of which were inserted five copies of the GAL4 DNA-binding motif. As a positive control, a GAL4 fusion was also made with amino acids 200–325 of c-Myb, a region known to contain a trans-activation domain (7). It was found that pGal/B-Myb(184–704) containing all B-Myb sequences downstream of the DNA-binding domain was unable to trans-activate the reporter (Figure 5B, column 2). Truncation of sequences by deletion from the C-terminus unmasked only 2-fold trans-activation with pGal/B-Myb(184–508) and further deletion with pGal/B-Myb(184–349) abrogated any trans-activation activity (Figure 5B, columns 3 & 4). Only with pGal/B-Myb(360–561) was significant trans-activation activity observed (Figure 5B, column 7); this plasmid trans-activated transcription of pUAScat by 9-fold, which is still weak in comparison to the c-Myb fusion which demonstrated 150-fold stimulation in this assay (Figure 6A, column 2). It is notable that B-Myb aa 360–561 encompasses the C-terminal sequence that shows homology with c-Myb and other family members (2,3).

As B-Myb was found to be able to repress c-Myb trans-activation of Myb-binding site containing promoters (Figure 4), it was investigated whether the Gal/B-Myb fusions would show a similar activity on trans-activation of pUAScat by the c-Myb domain. It was found that stimulation of pUAScat transcription by pGal/c-Myb(200–325) was markedly inhibited by certain Gal/B-Myb fusions (Figure 6). Most notably, co-transfection with pGal/B-Myb(227–349) inhibited this activity approximately 20-fold (Figure 6, column 4), while pGal/B-Myb(360–561) and pGal/B-Myb(561–704) inhibited trans-activation by 2.4-fold and 4.6-fold, respectively (Figure 6, columns 5 and 6). It is possible that variation in relative levels of expression of these proteins, which have not been determined, could account for the differences in inhibition observed. However, it is of interest that the most marked inhibition of c-Myb activity was obtained with B-Myb aa 227–349, wherein by reference to c-Myb it may have been expected that a transcription regulatory domain would be located.

Such a high level of repression by pGal/B-Myb(227–349) suggested a specific effect upon c-Myb domain trans-activation, rather than simply competition for GAL4 binding sites in

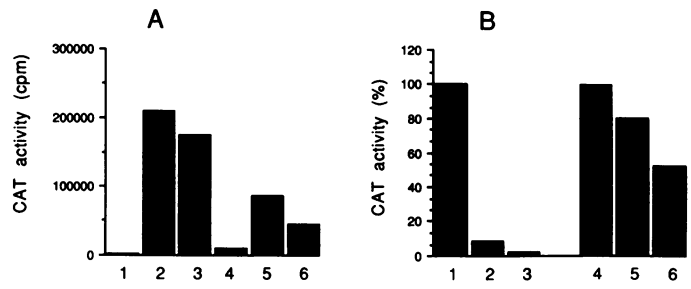

Figure 6. Specific inhibition of c-Myb trans-activation domain activity by B-Myb sequences. **(A)** CAT activity was assayed in extracts of NIH3T3 cells transfected with 4μg pUAScat together with 4μg of the empty vector pMG147Spe only (column 1) or 2μg pGal/c-Myb(200–325) plus 2μg of the following plasmids: pMG147Spe (column 2), pGal/B-Myb(184–226) (column 3), pGal/B-Myb(227–349) (column 4), pGal/B-Myb(360–561) (column 5) and pGal/B-Myb(561–704) (column 6). **(B)** CAT activity was assayed in extracts of NIH3T3 cells transfected with 4μg pUAScat together with 2μg of the control plasmid pMG147Spe (columns 1 and 4), 2μg pGal/B-Myb(227–349) (columns 2 and 5) or 4μg pGal/B-Myb(227–349) (columns 3 and 6) and either 2μg pGal/c-Myb(200–325) (columns 1–3) or pGal/VP16 (columns 4–6).

pUAScat or competition by hetero-dimerisation with Gal/c-Myb(200–325). To provide further evidence for this contention, the affects of Gal/B-Myb(227–349) on trans-activation of pUAScat by Gal/c-Myb(200–325) were compared with that on Gal/VP16, in which GAL4 was fused with a region of HSV-1 VP16 (aa 423–491) which contains a strong trans-activation sequence (22,23). It was found in this experiment that trans-activation by the VP16 domain was only modestly inhibited (2-fold) by co-transfection with 4μg pGal/B-Myb(227–349) (Figure 6, column 6), an effect that could be due to competition as noted above. In contrast, inhibition of trans-activation by the c-Myb domain was 50-fold with 4μg pGal/B-Myb(227–349) (Figure 6, column 3). This result is therefore consistent with the notion that B-Myb aa 227–349 specifically inhibited the ability of c-Myb to activate transcription.

DISCUSSION

It was found here, contrary to expectation, that mouse B-Myb, while containing a DNA-binding domain that could interact with a Myb DNA-binding motif, did not trans-activate SV40 or human c-*myc* promoters containing such binding sites. In contrast, it has been reported that human B-Myb shares the ability of mouse c-Myb to trans-activate these promoters (8,10). As the human B-Myb sequences responsible for trans-activation have not been mapped, the reason for this discrepancy is not clear. It should be noted, however, that the C-termini of the human and mouse proteins, while showing some regions of almost complete homology, also contain quite divergent sequences. Translation of B-Myb proteins *in vitro* suggest that the C-terminus of B-Myb may participate in negative regulation of binding to DNA (Figure 2). Presumably, this inhibition reflects modification of B-Myb by reticulocyte protein kinases as has been described for the *max* protein (24), and potentially could account for the inability to demonstrate trans-activation by mouse B-Myb. It is unclear whether such postulated modifications of B-Myb would necessarily occur *in vivo*, however. It seems inconceivable that B-Myb does not bind DNA in the cell, though it is possible that binding may be regulated for example at certain stages of the

cell-cycle. Furthermore, the observed antagonism of c-Myb trans-activation by B-Myb (Figure 4) suggests that the latter protein is competent to bind DNA *in vivo*. Additional experiments in which C-terminal sequences were fused to GAL4 indicate that mouse B-Myb lacks a strong trans-activation domain such as that present in c-Myb, pointing to fundamental differences in the composition of these related proteins that may more readily account for differences in their properties.

Whereas mouse B-Myb was inactive on the SV40 early and human c-*myc* promoters, it was able to weakly trans-activate the DPα promoter. The nature of this latter activity is unknown, but at least for c-Myb does not seem to involve sequence-specific DNA recognition or the recognised trans-activation domain (unpublished observations). Despite close correlation between the time of induction of B-*myb* and DPα gene expression late in G_1 following serum-stimulation of fibroblasts (3), we have observed that constitutive expression of a transfected B-*myb* gene in these cells did not affect the time of endogenous DPα gene induction (unpublished observations). It seems unlikely therefore that the activity demonstrated in co-transfection experiments infers a direct role for *myb* genes in DPα gene transcription.

It was of interest that not only did mouse B-Myb fail to trans-activate the SV40 early and human c-*myc* promoters, but it also inhibited the ability of c-Myb to do so. Although the evidence is indirect, experiments with GAL4 fusions (Figure 6) pointed to B-Myb aa 227–349 having a particular role in this inhibition. Indeed, preliminary studies with a C-terminally truncated mouse B-Myb protein comprising only aa 1–349 suggest that this protein is much more effective at this inhibitory activity than the full-length protein (unpublished observations). It is notable that mouse B-Myb aa 227–349 contains a 52 aa section that shows complete identity to the human homologue (3). Part of this highly conserved region has homology to a short A-Myb sequence that lies just downstream from its putative trans-activation domain. In some respects it is paradoxical that the section of B-Myb which appears to confer inhibition of c-Myb trans-activation occupies a position topologically equivalent to that within c-Myb (i.e. aa 275–325) previously demonstrated to comprise a trans-activation domain (7). This coincidence, however, may reflect a domain of these two related proteins that perform distinct functions in transcription regulation, for example by interacting with different components of the general transcription apparatus. Without access to information on such interactions it is difficult to explain why this should lead to the observed inhibitory effects of B-Myb on c-Myb, and clearly a more mechanistic approach to the activity of these proteins is required to address this issue.

It may be of some significance that the only region of mouse B-Myb shown to have any trans-activation activity when linked to GAL4 encompasses a region that is extensively conserved in the human homologue and moreover shows some homology with both c-Myb and A-Myb as well as the *Drosophila* Myb protein (2). While it would be dangerous to ascribe too much significance to the relatively weak activity seen with pGal/B-Myb(360–561) (Figure 5), this finding at least raises the possibility that this section of the Myb proteins has activity other than that of a negative regulatory domain (6).

Perhaps it should not be inferred from experiments reported here that B-Myb necessarily has no potential to act as a transcription activator: indeed it has some activity on the DPα promoter (Figure 1). Possibly in the appropriate context it may co-operate with factors bound to adjacent binding sites and thus potentiate promoter activation. What our findings do demonstrate, however, is that B-Myb can not be considered as a homologue of c-Myb. Clearly the properties of these related proteins differ, especially in relation to any inherent ability of these proteins to act as trans-activators. This implies that, although they may bind to the same or an overlapping set of target gene promoters, their ability to activate transcription as a result may be entirely different. Thus, they may have mutually antagonistic affects on trans-activation or, alternatively, may rely on co-operation with distinct transcription factors to exert their effect. If the latter possibility applies, the related Myb proteins may actually be implicated in regulation of rather different target genes. Such notions are consistent with the findings that both c-*myb* and B-*myb*, as well as A-*myb*, are co-expressed in a number of cell types (2,3,25).

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