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An inhibitory carboxyl-terminal domain in Ets-1 and Ets-2 mediates differential binding of ETS family factors to promoter sequences of the *mb-1* gene

(DNA-binding proteins/B-cell-specific gene expression)

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ABSTRACT **The *mb-1* gene is expressed only during the early stages of B-lymphocyte differentiation. Here we show that the *mb-1* proximal promoter region contains a functionally important binding site for members of the ETS family of DNA-binding proteins. We found that both the E26 virus-encoded v-ets and the myeloid/B-cell-specific factor PU.1 bind efficiently to this site *in vitro*. By contrast, Ets-1, the lymphocyte-specific cellular homologue of v-ets, and the related, more ubiquitously expressed Ets-2 protein interacted weakly with this binding site. DNA binding by both Ets-1 and Ets-2, however, could be increased 20- to 50-fold by deleting as few as 16 carboxyl-terminal amino acids. The inhibitory carboxyl-terminal amino acid sequence is highly conserved between Ets-1 and Ets-2 but is not present in either v-ets or PU.1. Replacement of the carboxyl-terminal amino acids of v-ets with those of Ets-1 decreased DNA binding by v-ets drastically. Cotranslation of Ets-1 transcripts encoding proteins of different lengths suggested that Ets-1 binds DNA as a monomer. Therefore, the carboxyl-terminal inhibitory domain appears to interfere directly with DNA binding and not with homodimerization. Finally, the functional relevance of ETS factor binding to the *mb-1* promoter site was evidenced by the stimulation of transcription through this site by a v-myb–v-ets fusion protein. Together, these data suggest that one or more ETS family factors are involved in the regulation of *mb-1* gene expression.**

The B-lymphocyte-specific *mb-1* gene is a model for the regulation of lineage- and stage-specific gene expression. The *mb-1* gene encodes a 32-kDa protein that is required for the expression of IgM molecules on the B-cell surface (for review, see ref. 1). Specific mb-1 transcripts have been found only in cells that comprise the early stages of B-lymphoid-cell differentiation (2). Tissue culture transfection experiments suggested that proximal *mb-1* promoter sequences residing between nucleotides −68 to +70 contain sufficient information for early B-cell-specific gene expression (3). Analysis of linker scanning and deletion mutations across this region indicated the importance of at least two distinct nuclear factor-binding sites for *mb-1* promoter function (3). Both sites were shown to interact with ubiquitous factors *in vitro*. One factor was identified as Sp1, whereas the identity of the second factor, termed MUF2 (*mb-1* upstream factor 2), is unknown. Interestingly, the MUF2 site is identical to a sequence within the polyomavirus enhancer region (PEA3 site) that is recognized by the nuclear DNA-binding protein Ets-1 *in vitro* (4). Ets-1, which is expressed primarily in lymphoid tissues, has been implicated as a tissue-specific regulator of transcription (for review, see ref. 5). Therefore, the question arises as to whether Ets-1 or another lymphoid-specific factor can bind the MUF2 site and participate in the regulation of *mb-1* gene expression.

The ETS family of nuclear proteins is characterized by a conserved DNA-binding motif referred to as the ETS domain (6). Two members of the ETS family, the B- and T-cell-specific Ets-1 and myeloid/B-cell-specific PU.1 proteins, are expressed in a cell-type-specific manner that suggests a role in the regulation of lymphoid-specific genes (7–11). By contrast, Ets-2, which is closely related to Ets-1, is expressed in a wide range of tissue types and cultured cells (8, 9, 12). The v-ets polypeptide is encoded by a transduced c-*ets-1* gene expressed by the avian retrovirus E26 as part of a tripartite fusion protein, $p135^{gag\text{-}myb\text{-}ets}$ (13, 14).

Here we report that Ets-1 and PU.1 bind the *mb-1* promoter *in vitro*, although with different efficiencies. We show that this differential DNA binding is governed by the presence of inhibitory carboxyl-terminal amino acids in Ets-1 and Ets-2, which are absent in PU.1 or v-ets.

MATERIALS AND METHODS

Plasmid Construction and *in Vitro* Mutagenesis. Plasmids pv-etsHS4 and pv-etsHB2 contain either the *Hpa* I–*Stu* I or *Hpa* I–*Bgl* I fragments of pc-etsm (provided by J. M. Bishop, University of California, San Francisco) for expression *in vitro* (15). The murine c-ets-1 cDNA clone SKc-ets1.6 (16) was provided by J. H. Chen (M.D. Anderson Cancer Center, Houston). The murine Ets-2 (c-ets2-KS+) and PU.1 (PU.1-KS+) cDNA clones were provided by R. Maki (LaJolla Cancer Research Center). Mutations were introduced by using oligonucleotide-directed mutagenesis (3) and primers: pc-etsN385D, 5′-CAGTTTCTCATAATCCATCTTAGGTTTGT; c-etsI401V, 5′-CGTCTTGTGGATGACATTTTTGTCATAATAGTAGCG; pc-etsCΔ1, 5′-CGTGCAGCTCTTCAGGGATCGATCACAGCAGGCTCTGCAGG; and PU.1ΔC, 5′-GGCCAGGCCCCCACGTC- ACTGCAGCTCGCCGCTGAACTGG. The plasmid pEts-1/v-ets was constructed by insertion of the *Hin*dIII–*Sal* I fragment of pc-etsm into SKc-ets1.6, and pv-ets/Ets-1 was made by insertion of the *Eco*47III–*Not* I fragment of SKc-ets1.6 into pv-etsHS4. pc-etsHCΔ (to make Ets-1ΔNΔC; see Fig. 4) was constructed by deletion of the *Hpa* I fragment from pc-etsCΔ1. EVE26S3 and EVE26A2 were made by subcloning the 2.2-kilobase (kb) *Eco*RI fragment of pc-etsm into EVRF2 (17).

***In Vitro* Transcription, Translation, and Protein Analysis.** Plasmid DNA was transcribed *in vitro*, and RNA transcripts were purified with Qiagen columns as suggested by the manufacturer (Qiagen, Chatsworth, CA). Ets-2ΔC protein was translated from RNA transcribed from c-ets2-KS+ DNA

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Abbreviations: wt, wild type; CAT, chloramphenicol acetyltransferase; MUF2, *mb-1* upstream factor 2.

linearized with *Bsp*EI before transcription. Synthetic RNA (1 μg) was used in a 25-μl translation reaction with nuclease-treated rabbit reticulocyte lysate (Promega) and unlabeled or ^{35}S-labeled (Amersham) methionine at 30°C. ^{35}S-labeled proteins were analyzed by using SDS/PAGE and autoradiography as described (15). Quantitation of labeled protein was done by using a Molecular Dynamics (Sunnyvale, CA) PhosphorImager system and was adjusted for the number of methionines per polypeptide.

DNA Binding and Electrophoretic Mobility-Shift Assays. DNA-binding assays were performed as described (3), except that sheared salmon testes DNA at 20 μg/ml was included. Probe DNA was incubated without or with competitor oligonucleotides together with labeled probe and 2 μl of translation products for 30 min at 20°C.

Transfection Assays. LMtk$^-$ cells were transfected by using the DEAE-dextran/chloroquine method (18) with 2 μg of total DNA and harvested 42 hr after transfection. Chloramphenicol acetyltransferase (CAT) and luciferase assays were done and quantified as described (3).

RESULTS

v-ets Specifically Binds Sequences Within the *mb-1* Promoter. We have shown (3) that the nucleotide sequence between −55 and −47 of the *mb-1* promoter is important for promoter function. Moreover, this site is bound *in vitro* by a ubiquitous factor, termed MUF2, detected in nuclear extracts (3). Examination of the nucleotide sequence of the MUF2 site (Fig. 1*A*) revealed a 9/9-base-pair (bp) identity with a known binding site (5′-CAGGAAGTG, termed the PEA3 site) for the nuclear DNA-binding proteins Ets-1 and Ets-2 in the polyomavirus enhancer (20). To determine whether this sequence can also serve as a binding site for Ets-1-related proteins, we performed electrophoretic mobility-shift assays with a ^{32}P-labeled duplex oligonucleotide containing the MUF2 site (Fig. 1*A*, wt) and *in vitro*-translated Ets-1, Ets-2, PU.1, and v-ets polypeptides. Preliminary data suggested that each of these factors bound the DNA probe specifically, although large differences in the level of factor binding were observed (data not shown). The highest levels of DNA binding were seen with a polypeptide composed of only the v-ets segment (amino acids 85–491 of v-ets; ref. 13) of the p135$^{gag-myb-ets}$ protein (Fig. 1*B*, lane 2). DNA binding by v-ets was inhibited by excess unlabeled wt (lanes 3 and 4) and mutated mut1 (lanes 9 and 10) oligonucleotides. By contrast, the sequences mutated in oligonucleotides mut2 (lanes 7 and 8) and mut4 (lanes 5 and 6) are important for binding by v-ets. An oligonucleotide containing the binding site for Ets-1 identified within the T-cell receptor α enhancer (19) competed less efficiently for v-ets binding than did the wt MUF2 site (lanes 11 and 12).

Differential DNA Binding by v-ets Versus Ets-1. Because v-ets and Ets-1 are nearly identical within their ETS domains (83 of 85 amino acids are identical; a partial sequence is shown in Fig. 2*A*), we expected that the cellular homologue Ets-1 would bind this nucleotide sequence similarly. Ets-1 specifically bound the MUF2 site; however, the level of binding was 50-fold lower than that seen for v-ets (Fig. 2*B*, lanes 2 and 3) for similar amounts of protein (Fig. 2*B*, *Lower*).

To determine whether the relative DNA binding of Ets-1 and v-ets is influenced by the two amino acid differences between their respective ETS domains, oligonucleotide-directed mutagenesis of Ets-1 was done to convert one or both of these amino acids to those present in v-ets (Fig. 2*A*). The substitution of aspartate for asparagine at position 385 (numbered using the sequence of Chen, ref. 16) reduced DNA binding to nearly undetectable levels (Fig. 2*B*, lane 4). The substitution of valine for isoleucine at position 401 also reduced binding by a factor of 20 (lane 5), and mutation of both residues did not increase DNA-binding activity (lane 6).

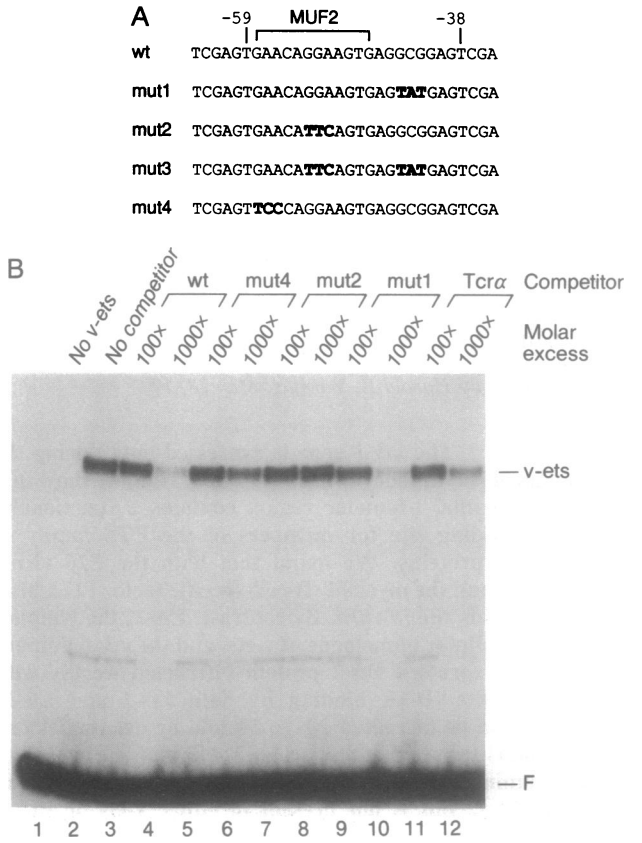

FIG. 1. Specific binding of the *mb-1* promoter proximal region by v-ets *in vitro*. (*A*) Wild-type (wt) and mutant (mut) oligonucleotide sequences. Oligonucleotides include nucleotides −59 to −38 of the *mb-1* promoter region as shown. Mutations are indicated in boldface letters. The MUF2-binding site is indicated. Electrophoretic mobility-shift assay of v-ets binding was done with the ^{32}P-labeled wt oligonucleotide in the absence or presence of unlabeled competitor DNA and *in vitro*-translated v-ets. The murine T-cell receptor α enhancer oligonucleotide has been described (19). F, free probe.

Therefore, the two mutations are not the only residues responsible for the differential DNA binding by the two proteins. Additional amino acid differences in more amino-terminal domains may compensate for the negative effects of D385 and V401 on DNA binding.

To examine whether the divergent carboxyl termini of the two proteins (Fig. 2*A*) govern the differences in DNA binding, we constructed plasmids for the expression of chimeric Ets-1/v-ets polypeptides *in vitro*. Substitution of the 16 carboxyl-terminal amino acids of v-ets for the 12 terminal amino acids of Ets-1 dramatically increased the level of DNA binding relative to Ets-1 (Fig. 2*B*, lane 7). To test whether the carboxyl-terminal sequence of v-ets directly participates in DNA binding, we deleted 22 amino acids from the carboxyl terminus of the protein. The shortened v-ets protein bound the mb-1 site slightly better than did v-ets with the complete carboxyl terminus (65- vs. 53-fold relative to Ets-1; lane 8 vs. 2), suggesting that the v-ets carboxyl terminus is not required for DNA binding. Insertion of a translation termination codon in place of the glycine residue at position 423 of Ets-1, resulting in the deletion of 18 amino acids (Fig. 2*A*), enhanced the DNA binding of the Ets-1ΔC polypeptide 36-fold relative to native Ets-1 (Fig. 2*B*, lane 9). These results suggested that the enhanced binding of the chimeric Ets-1/v-ets polypeptide was due to deletion (or replacement) of the normal Ets-1 carboxyl terminus. To further substantiate this hypothesis, we replaced the normal carboxyl terminus of v-ets with that of Ets-1 (v-ets/Ets-1 in Fig. 2*A*). Binding of this chimeric

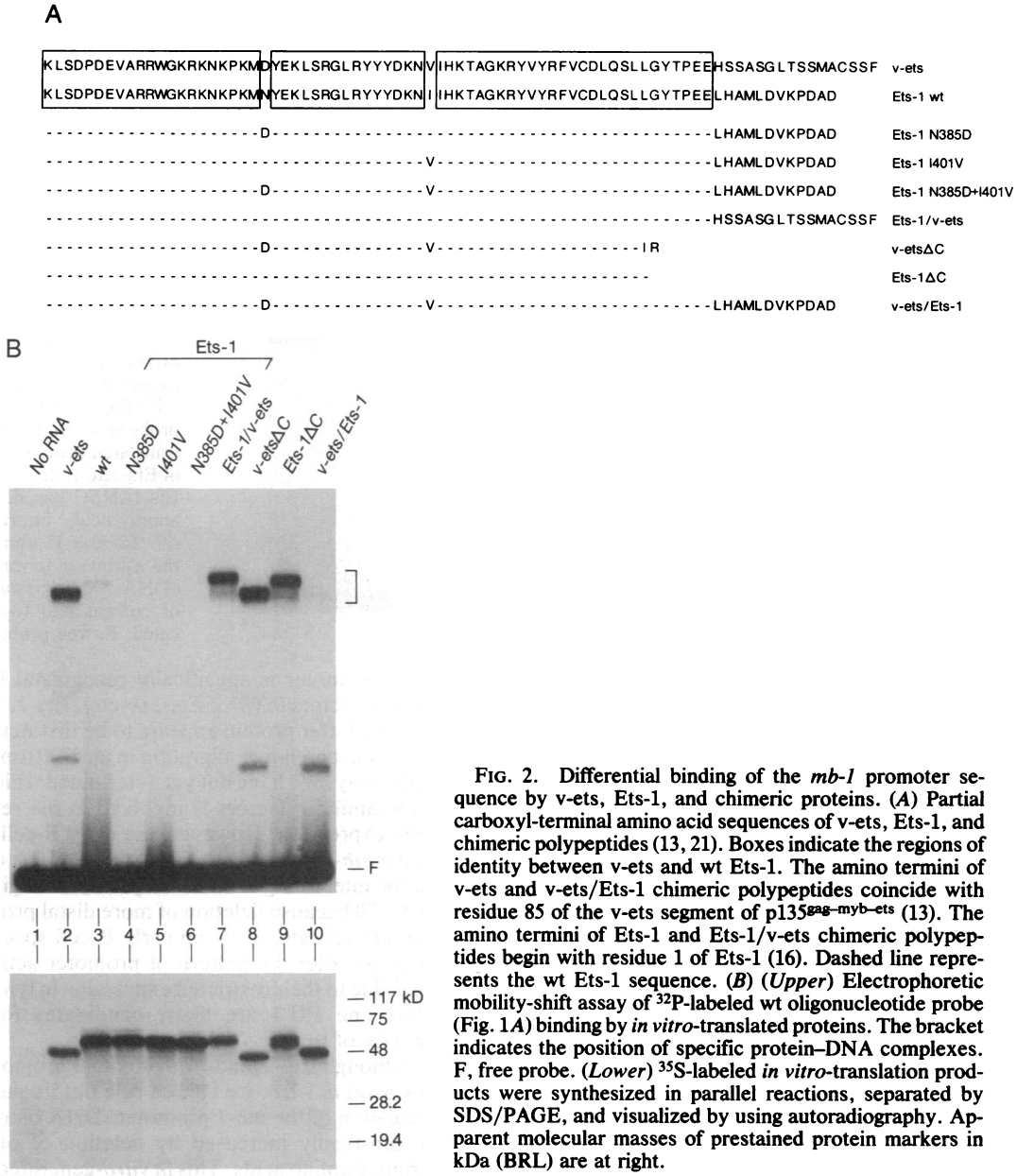

FIG. 2. Differential binding of the *mb-1* promoter sequence by v-ets, Ets-1, and chimeric proteins. (*A*) Partial carboxyl-terminal amino acid sequences of v-ets, Ets-1, and chimeric polypeptides (13, 21). Boxes indicate the regions of identity between v-ets and wt Ets-1. The amino termini of v-ets and v-ets/Ets-1 chimeric polypeptides coincide with residue 85 of the v-ets segment of $p135^{gag-myb-ets}$ (13). The amino termini of Ets-1 and Ets-1/v-ets chimeric polypeptides begin with residue 1 of Ets-1 (16). Dashed line represents the wt Ets-1 sequence. (*B*) (*Upper*) Electrophoretic mobility-shift assay of ^{32}P-labeled wt oligonucleotide probe (Fig. 1*A*) binding by *in vitro*-translated proteins. The bracket indicates the position of specific protein–DNA complexes. F, free probe. (*Lower*) ^{35}S-labeled *in vitro*-translation products were synthesized in parallel reactions, separated by SDS/PAGE, and visualized by using autoradiography. Apparent molecular masses of prestained protein markers in kDa (BRL) are at right.

protein was reduced by a factor of 34 relative to that of v-ets (Fig. 2*B*, lane 10). The data indicate that the 12 carboxyl-terminal amino acids of Ets-1 strongly suppress DNA binding by both Ets-1 and the closely related v-ets.

To examine whether the Ets-1 carboxyl terminus can inhibit the binding of a more distantly related factor, we substituted the 15 carboxyl-terminal amino acids of PU.1 with the 23-carboxyl-terminal amino acids of Ets-1. The Ets-1 carboxyl terminus decreased DNA binding by the chimeric protein by only 2- to 3-fold relative to wt PU.1 (data not shown). The Ets-1 carboxyl terminus appears to affect DNA binding only when linked to amino acids that are conserved between Ets-1 and v-ets.

Inhibition of Ets-2 DNA Binding by Its Carboxyl Terminus. The ETS domains of Ets-1 and Ets-2 are nearly identical (96% identity between the two murine ETS domains; ref. 6). A comparison of Ets-1 and Ets-2 carboxyl termini shows that the overall amino acid sequences flanking the ETS domains are highly conserved (9, 21). Therefore, we examined whether deletion of the 16 carboxyl-terminal amino acids of Ets-2 has an effect on DNA binding. For comparison, 13 amino acids were deleted from the carboxyl terminus of PU.1, which shares 38% of the ETS-domain amino acids with murine Ets-1 (6). Ets-2 and PU.1 proteins each bound the MUF2 site specifically *in vitro* (Fig. 3; data for specific competition are not shown). Similar to Ets-1, deletion of the carboxyl terminus enhanced DNA binding by 20-fold (lanes 2 and 3). In contrast, DNA binding by PU.1 was more efficient than by native Ets-1, and deletion of PU.1 carboxyl-terminal sequences did not further enhance DNA binding (lanes 5 and 6).

Stoichiometry of Ets-1–DNA Complexes. The inhibition of DNA binding by the carboxyl termini of Ets-1 and Ets-2 could involve the regulation of homodimer or multimer formation. To determine whether Ets-1 binds the MUF2 site as a monomer or homodimer, Ets-1ΔC polypeptide lacking the inhibitory carboxyl terminus or Ets-1ΔNΔC polypeptide with the additional deletion of amino acids 37–231 was synthesized either separately or together *in vitro*. Similar amounts of translated proteins were synthesized in each reaction (data not shown). When analyzed in an electrophoretic mobility-shift assay, Ets-1ΔC/DNA complexes migrated more slowly than complexes containing the ΔNΔC polypeptide (Fig. 4, lane 2 vs. 4). Cotranslation of the ΔC and ΔNΔC transcripts

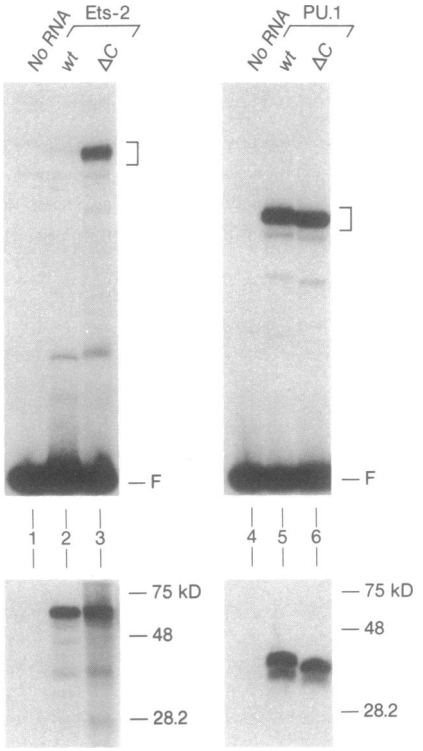

FIG. 3. DNA binding by the nuclear factor Ets-2 but not by the related factor PU.1 is enhanced after deletion of carboxyl-terminal sequences. (*Upper*) Electrophoretic mobility-shift assay of ^{32}P-labeled wt oligonucleotide probe (Fig. 1*A*) binding by *in vitro*-translated proteins. wt, Full-length polypeptides. Ets-2ΔC has a deletion of 16 carboxyl-terminal amino acids, and similarly, PU.1ΔC has a deletion of 13 amino acids from its carboxyl terminus. The brackets indicate the position of specific protein–DNA complexes. F, free probe. (*Lower*) ^{35}S-labeled *in vitro*-translation products were synthesized in parallel reactions, separated by SDS/PAGE, and visualized by autoradiography. Apparent molecular masses of prestained protein markers in kDa (BRL) are at right.

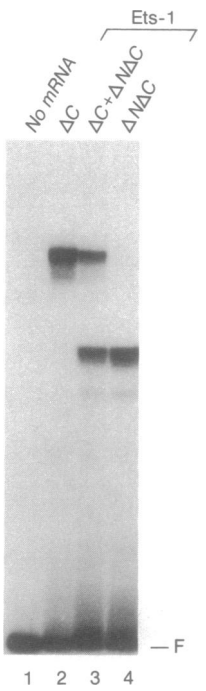

FIG. 4. Ets-1 binds the *mb-1* promoter site as a monomer. Electrophoretic mobility-shift assay of ^{32}P-labeled wt oligonucleotide probe (Fig. 1*A*) binding by *in vitro*-translated proteins. The structure of Ets-1ΔC is described in Fig. 2*A*. Ets-1ΔNΔC has deletions of 196 amino acids internally (37–231; ref. 16) and 12 amino acids from the carboxyl terminus. The two cDNAs were translated separately or cotranslated together as indicated. F, free probe.

did not result in the appearance of any complexes with intermediate mobility (lane 3), suggesting that Ets-1 binds DNA as a monomer.

Transactivation of Gene Expression via the *mb-1* Promoter ETS Binding Site. The functional relevance of ETS factor binding to the MUF2 site was examined by cotransfecting reporter plasmids, which contain three tandem wt or mutated *mb-1* promoter MUF2 sites adjacent to the c-*fos*/CAT gene (3), together with the E26 v-*myb*/v-*ets* gene under the control of the cytomegalovirus promoter/enhancer (Fig. 5). The Sp1 binding site of the *mb-1* promoter, which is normally adjacent to the MUF2-binding site, was mutated in both reporter plasmids to limit the analysis to interactions with the MUF2 site. The polypeptide encoded by the activator protein does not include the v-*myb* DNA-binding domains (22) but does contain a v-*myb* transcriptional activation domain (23). Transcriptional activation (12-fold; lane 2 vs. 3) by the v-myb–v-ets fusion protein was observed only when the wt MUF2 site was present in the reporter gene and only when the v-*myb*–v-*ets* fusion gene was expressed in the sense orientation. Therefore, the MUF2 site in the *mb-1* promoter can act *in vivo* as a functional binding site for ETS factors to activate transcription. In contrast, little or no activation was seen when the effector plasmids expressed other ETS family factors, including full-length Ets-1 and PU.1 or the carboxyl-terminally truncated Ets-1ΔC (data not shown).

DISCUSSION

We have shown that the functionally important nucleotide sequence 5′-GAACAGGAAGTG in the early B-cell-specific *mb-1* promoter is specifically recognized by four different nuclear factors *in vitro*: Ets-1 (v-ets), Ets-2, PU.1, and MUF2 (3). The latter protein appears to be distinct from the others, based on its relative migration in an electrophoretic mobility-shift assay. We have not yet determined which member of the ETS family of factors is involved in the regulation of *mb-1* gene expression. However, the early B-cell-specific activity of the *mb-1* promoter is likely to involve a cell-type-specific factor interacting within the promoter region between −68 and +70 because deletion of more distal promoter sequences that are recognized by an early B-cell-specific factor (EBF) does not alter the pattern of promoter activity (24). Therefore, due to their restricted expression in lymphoid cells, both Ets-1 and PU.1 are likely candidates for regulating the activity of the *mb-1* promoter region.

Although Ets-1 binds the proximal promoter sequence at a low level *in vitro*, we cannot rule out its putative role in the regulation of the *mb-1* promoter. DNA binding by Ets-1 can be drastically increased by deletion of only 18 carboxyl-terminal amino acids. This *in vitro*-generated enhancement of DNA binding may reflect a mechanism that normally antagonizes the action of the carboxyl-terminal inhibitory domain *in vivo*. Such a change in the ability to bind DNA could result from posttranslational modifications of amino acids involved in the inhibition of DNA binding. In this regard, nuclear Ets-1 is extensively phosphorylated in activated T (25, 26) and B lymphocytes (27).

Alternatively, the binding of Ets-1 and/or Ets-2 may be potentiated by dimerization with a second DNA-binding protein. Heterodimerization could increase the DNA-binding affinity of Ets-1 by protein–protein interactions and/or may antagonize the inhibitory effect of the carboxyl terminus by inducing a conformational change in Ets-1. Heteromeric complex formation between ETS family members and other factors has been observed. (*i*) The binding of Elk-1 (28) and SAP1 (29) to nucleotide sequences that regulate the c-*fos* promoter depends upon the interaction of these proteins with serum response factor and DNA in a ternary complex, and deletion of an internal domain from SAP1 enhances the ability of this protein to bind DNA in the absence of serum response factor (29). (*ii*) PU.1 may interact with a factor(s) present in myeloma cells because the mobility of PU.1–DNA complexes formed with a sequence within the κ light chain 3′

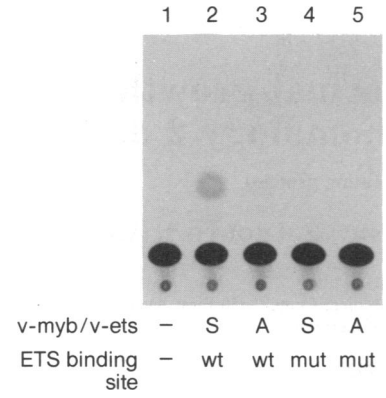

FIG. 5. The MUF2 site in the *mb-1* promoter mediates transactivation by v-myb–v-ets protein. Three tandem copies of the mut1 oligonucleotide (see Fig. 1*A*), which contains a wt MUF2 site and a mutated Sp1-binding site, were inserted immediately upstream of the c-*fos* minimal promoter linked to the CAT reporter gene (3). As a control, the mut3 oligonucleotide, which contains mutations in both sites (see Fig. 1*A*), was used to construct a mutant reporter plasmid. wt and mutant (mut) reporter plasmids were transfected together with an effector plasmid encoding a v-myb–v-ets fusion protein (178 carboxyl-terminal amino acids of v-myb fused to the entire 491 amino acids of v-ets) expressed in the sense (S) or antisense (A) orientation from the cytomegalovirus enhancer/promoter. A Rous sarcoma virus–luciferase plasmid was included as a control for transfection efficiency. Cell lysates were prepared 42 hr after transfection, lysates for CAT assays were normalized for luciferase activity, and CAT activity was quantitated by using the PhosphorImager.

enhancer region is altered by the addition of myeloma nuclear extract (30). (*iii*) The mammalian transcription factor GABP is composed of the ETS-related GABPα and notch-related GABPβ subunits (31). Using the *mb-1* proximal promoter region (-59 to -38) as a probe, we have not observed any changes in the mobility of protein–DNA complexes after adding early B-cell nuclear extracts to binding reactions containing the ETS family factors described in this study (data not shown). However, the interaction of the ETS factors with nuclear proteins may not be readily detectable because of their low abundance.

The carboxyl terminus of Ets-1 could inhibit DNA binding by a number of different mechanisms. The carboxyl terminus could interact directly with amino acids that contact DNA. In a variation of this model, the carboxyl terminus could interact with another region of the polypeptide. Indeed, a second internal inhibitory domain was recently identified in Ets-1, suggesting that binding is inhibited by specific intramolecular contacts between both domains (32). This result is consistent with our observation that a peptide containing the carboxyl-terminal 15 amino acids of Ets-1 did not inhibit DNA binding when incubated with Ets-1ΔC (lacking these same amino acids) at peptide concentrations up to 1 mM (data not shown). Moreover, the carboxyl terminus of Ets-1 had only a minor effect on the DNA binding of PU.1/Ets-1 chimeric polypeptides, although PU.1 and Ets-1 recognize similar DNA sequences.

Of the ETS family factors tested so far, only the v-myb–v-ets fusion protein was able to appreciably transactivate transcription through the MUF site. We suspect that the relatively high binding affinity of v-ets for this site, together with the presence of two putative activation domains (one in the carboxyl-terminal domain of v-myb and one in the amino-terminal domain of v-ets; refs. 23 and 32), accounts for this effect. As previously suggested, the inability of the cellular ETS proteins to activate transcription in similar assays may reflect the need for interactions with other transcription factors bound to the promoter. Studies of ETS family proteins and other factors bound to the *mb-1* promoter may explain how a specific member of a family of nuclear factors is recruited for the activation of transcription in early B cells.

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1. Cambier, J. C. (1991) *Curr. Opin. Immunol.* **1,** 25–27.
2. Sakaguchi, N., Kashiwamura, S.-i., Kimoto, M., Thalmann, P. & Melchers, F. (1988) *EMBO J.* **7,** 3457–3464.
3. Travis, A., Hagman, J. & Grosschedl, R. (1991) *Mol. Cell. Biol.* **11,** 5756–5766.
4. Wasylyk, B., Wasylyk, C., Flores, P., Begue, A., Leprince, D. & Stehelin, D. (1990) *Nature (London)* **346,** 191–193.
5. Leiden, J. M. (1992) *Immunol. Today* **13,** 22–30.
6. Karim, F. D., Urness, L. D., Thummel, C. S., Klemsz, M. J., McKercher, S. R., Celada, A., Van Beveren, C., Maki, R. A., Gunther, C. V., Nye, J. A. & Graves, B. J. (1990) *Genes Dev.* **4,** 1451–1453.
7. Ghysdael, J., Gegonne, A., Pognonec, P., Dernis, D., Leprince, D. & Stehelin, D. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1714–1718.
8. Bhat, N. K., Fisher, R. J., Fujiwara, S., Ascione, R. & Papas, T. S. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3161–3165.
9. Watson, D. K., McWilliams, M. J., Lapis, P., Lautenberger, J. A., Schweinfest, C. W. & Papas, T. S. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 7862–7866.
10. Leprince, D., Gesquiere, J. C. & Stehelin, D. (1990) *Oncogene Res.* **5,** 255–265.
11. Klemsz, M. J., McKercher, S. R., Celada, A., Van Beveren, C. & Maki, R. A. (1990) *Cell* **61,** 113–124.
12. Boulukos, K. E., Pognonec, P., Begue, A., Galibert, F., Gesquiere, J. C., Stehelin, D. & Ghysdael, J. (1988) *EMBO J.* **7,** 697–705.
13. Nunn, M. F., Seeburg, P. H., Moscovici, C. & Duesburg, P. H. (1983) *Nature (London)* **306,** 391–395.
14. Leprince, D., Gegonne, A., Coll, J., de Taisne, C., Schneeburger, A., Lagrou, C. & Stehelin, D. (1983) *Nature (London)* **306,** 395–397.
15. Travis, A., Amsterdam, A., Belanger, C. & Grosschedl, R. (1991) *Genes Dev.* **5,** 880–894.
16. Chen, J. H. (1990) *Oncogene Res.* **5,** 277–285.
17. Matthias, P., Muller, M. M., Schreiber, E., Rusconi, S. & Schaffner, W. (1989) *Nucleic Acids Res.* **17,** 6418.
18. Grosschedl, R. & Baltimore, D. (1985) *Cell* **41,** 885–897.
19. Ho, J.-C., Bhat, N. K., Gottschalk, L. R., Lindstein, T., Thompson, C. B., Papas, T. S. & Leiden, J. M. (1990) *Science* **250,** 814–818.
20. Martin, M. E., Piette, J., Yaniv, M., Tang, W. J. & Folk, W. R. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 5839–5843.
21. Gunther, C. V., Nye, J. A., Bryner, R. S. & Graves, B. J. (1990) *Genes Dev.* **4,** 667–679.
22. Klempnauer, K.-H. & Sippel, A. E. (1987) *EMBO J.* **6,** 2719–2725.
23. Weston, K. & Bishop, J. M. (1989) *Cell* **58,** 85–93.
24. Hagman, J., Travis, A. & Grosschedl, R. (1991) *EMBO J.* **10,** 3409–3417.
25. Pognonec, P., Boulukos, K. E., Gesquiere, J. C., Stehelin, D. & Ghysdael, J. (1988) *EMBO J.* **7,** 977–983.
26. Koizumi, S., Fisher, R. J., Fujiwara, S., Jorcyk, C., Bhat, N. K., Seth, A. & Papas, T. (1990) *Oncogene* **5,** 675–681.
27. Fisher, C. L., Ghysdael, J. & Cambier, J. (1991) *J. Immunol.* **146,** 1743–1749.
28. Hipskind, R. A., Rao, V. N., Mueller, C. G. F., Reddy, E. S. P. & Nordheim, A. (1991) *Nature (London)* **354,** 531–534.
29. Dalton, S. & Treisman, R. (1992) *Cell* **68,** 597–612.
30. Pongubala, J. M. R., Nagulapalli, S., Klemsz, M. J., McKercher, S. R., Maki, R. A. & Acthison, M. L. (1992) *Mol. Cell Biol.* **12,** 368–378.
31. LaMarco, K., Thompson, C. C., Byers, B. P., Walton, E. M. & McKnight, S. L. (1991) *Science* **253,** 789–791.
32. Lim, F., Kraut, N., Frampton, J. & Graf, T. (1992) *EMBO J.* **11,** 643–652.