Communication

Histone H1 Enhances the DNA Binding Activity of the Transcription Factor EmBP-1*

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Previous work indicated that nuclear extracts isolated from embryogenic rice suspension cells treated with the phytohormone abscisic acid (ABA) have enhanced binding activity to an ABA response element (Em1a) in the promoter of the *Em* gene from wheat. We identified an activity in wheat and maize nuclear extracts that enhances binding of the recombinant transcription factor EmBP-1 to Em1a by 80-fold. Fractionation of nuclear extracts led us to identify histone H1 and HMGb (but not HMGc or -d) as two factors that can enhance the ability of EmBP-1 to bind to Em1a and account for at least a part of this activity of nuclear extracts. Our results, which indicate for the first time that histone H1 possesses this type of activity, lend further support to the model that positively charged proteins can drastically affect the DNA binding activity of specific transcription factors. Furthermore, our study points to these chromosomal proteins as potential targets of an ABA-mediated modification (*e.g.* acetylation) that could affect the regulation of *Em* gene expression.

The Em protein from a number of plants accumulates to high levels exclusively in the embryo during the maturation stage of seed development. Genetic and biochemical studies have shown that the presence of the phytohormone abscisic acid (ABA)[1] and the product of the *viviparous-1* (*vp1*) locus in maize (or its homolog in *Arabidopsis*, *abi3*) are required for expression of the *Em* gene (1, 2). A transient assay utilizing embryogenic rice protoplasts has been used to identify *cis* elements in the promoter of *Em* that are responsive to both ABA and VP1. These studies showed that a 76-base pair sequence (Region I) from the *Em* promoter is required for ABA-inducible expression and is involved in VP1 transactivation (3–5). Region I contains two elements encompassing the core sequence CACGTG (Em1a and Em1b), and mutations in either Em1a or Em1b drastically reduce the ability of ABA and VP1 to stimulate transcription. Tetramers of a 24-base pair sequence containing either the Em1a or Em1b elements fused to a non-responsive viral promoter can confer ABA and VP1 responsiveness (5).

A wheat embryo cDNA expression library was screened for proteins that bind to Region I. A basic leucine zipper protein, EmBP-1, was identified (6). Using bacterially expressed EmBP-1 and competition experiments in electrophoretic mobility shift assays, it was shown that EmBP-1 recognizes the Em1a and Em1b elements within Region I (6). Mutations in the CACGTG core of either sequence reduce expression in the transient assay and eliminate EmBP-1 binding. Furthermore, nuclear extracts isolated from embryogenic rice suspension cells treated with ABA have enhanced Region I binding activity compared with nuclear extracts from untreated cells (6). This result suggests that enhanced transcription from the Em promoter induced by ABA treatment could be the result of enhanced transcription factor binding to Region I. Recently, recombinant VP1 fusion protein has been shown to enhance the binding of EmBP-1 to Region I *in vitro* (7).

The goal of this study was to identify factors in nuclear extracts that alter the ability of EmBP-1 to bind to the Em1a element in the *Em* promoter. We identified an activity in wheat and maize nuclear extracts that enhances binding of recombinant EmBP-1 to Em1a by 80-fold. Fractionation of nuclear extracts led us to identify histone H1 and HMGb as being two factors that can enhance the ability of EmBP-1 to bind to Em1a and account for at least a part of this activity of nuclear extracts. Enhancement of EmBP-1 binding by histone H1 represents a novel activity for this protein.

EXPERIMENTAL PROCEDURES

Recombinant Protein Preparation—The plasmid pAN11, encoding recombinant maltose binding protein (MBP)/EmBP-1 (7) was transformed into *Escherichia coli* PR745 (New England Biolabs). One-liter cultures were grown to an A_{600} of approximately 0.8 and induced for 2 h at 37 °C with 0.3 mM isopropyl-1-thio-β-D-galactopyranoside. All subsequent steps were carried out at 4 °C. Cells were harvested, washed once with 100 ml of NCB (20 mM Tris, pH 7.5, 200 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol), and resuspended in 20 ml of NCB. A soluble protein extract was prepared by lysing the cells in a French press at 10,000 p.s.i. followed by centrifugation at 27,000 $\times$ *g*, 4 °C for 20 min. Contaminating *E. coli* genomic DNA was removed by precipitation with 0.48 ml of 10% polyethyleneimine followed by centrifugation as before. Recombinant MBP fusion protein was then purified on a 5-ml amylose resin column as recommended by the manufacturer (New England Biolabs). Purified fusion protein concentrations were determined using the Bio-Rad protein assay and band intensity on Coomassie Blue-stained gels.

Purified wheat histone H1 and HMG proteins were obtained as described previously (8, 9). Concentration of these proteins was determined by comparing band intensities of Coomassie Blue-stained gels with standards.

Preparation of Nuclear Extracts—Mature wheat embryos were isolated by flotation of wheat germ (General Mills) on 1.7 M sucrose, while maize embryos (both wild type and *vp1* mutant) were dissected from developing seeds at 20 days after pollination. Tissues were frozen in liquid nitrogen and stored at −70 °C until extraction. Tissues (2–5 g) were ground to a fine powder over liquid nitrogen and dispersed in 15 ml of Solution A (10 mM NaCl, 10 mM MES, pH 6.0, 5 mM EDTA, 0.6% Triton X-100, 0.25 M sucrose, 0.15 mM spermine, 0.5 mM spermidine, 20 mM β-mercaptoethanol, 0.2 mM PMSF). Nuclei were isolated by centrifugation at 1935 $\times$ *g* for 10 min at 4 °C, and the pellet was washed 2 times with 5 ml of Solution A followed by centrifugation as before. The nuclei were then resuspended with a Dounce homogenizer in 0.5 ml of Solution C (20 mM HEPES, pH 7.9, 25% glycerol, 420 mM NaCl, 1.5 mM MgCl$_2$, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM PMSF), and the soluble proteins were extracted at 4 °C for 2 h. The insoluble material

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[1] The abbreviations used are: ABA, abscisic acid; MBP, maltose binding protein; MES, 4-morpholineethanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride; HMG, high mobility group.
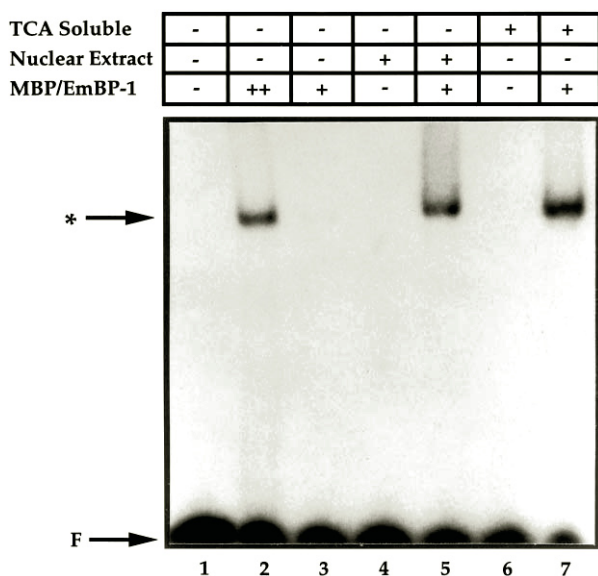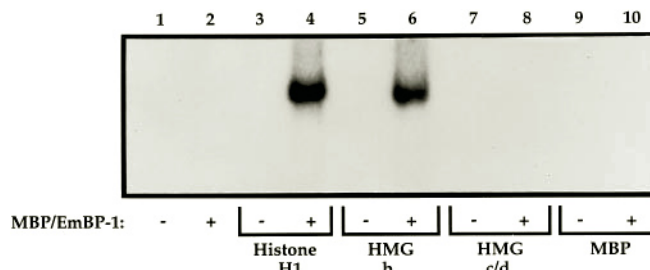
FIG. 1. **A trichloroacetic acid (*TCA*)-soluble fraction from nuclear extracts enhances the DNA binding activity of MBP/EmBP-1.** Electrophoretic mobility shift assay was performed using labeled Em1a as a probe. *Lanes*: *1*, free probe; *2*, 50 ng of MBP/EmBP-1; *3*, 9 ng of MBP/EmBP-1; *4*, 450 ng of wheat embryo nuclear extract; *5*, 450 ng of wheat embryo nuclear extract + 9 ng of MBP/EmBP-1; *6*, 1 μl of 2% trichloroacetic acid-soluble fraction of wheat embryo nuclear extract; *7*, 1 μl of 2% trichloroacetic acid-soluble extract of wheat embryo nuclear extract + 9 ng of MBP/EmBP-1. *F* denotes free probe, and the *asterisk* denotes retarded Em1a bound to MBP/EmBP-1.

was removed by centrifugation at $14{,}500 \times g$ for 15 min at 4 °C. The supernatant was dialyzed against 500 ml of Solution D (20 mM HEPES, pH 7.9, 20% glycerol, 100 mM KCl, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM PMSF), and the protein concentration was determined by Bio-Rad protein assay using bovine serum albumin as a standard.

Electrophoretic Mobility Shift Assay—Em1a probe was prepared by annealing two oligonucleotides (5′-GCGCTCGAGTGCCGGACACGT-GGC-3′ and 5′-CGCGTCGACGTCGCGCCACGTGTC-3′) and labeled by a fill-in reaction with [^{32}P]dCTP. The indicated amounts of proteins and extracts were incubated with 0.2–1 ng of labeled Em1a probe (10,000–20,000 cpm/reaction) for 10 min at room temperature. The binding reactions were carried out in 12 mM Tris, pH 7.9, 12% glycerol, 35 mM KCl, 0.07 mM EDTA, 1 mM dithiothreitol, 7.5 mM MgCl$_2$ and contained 2 μg of acetylated bovine serum albumin and 250 ng of poly(dI·dC) (Boehringer Mannheim) in a volume of 20 μl unless indicated otherwise. Binding reactions were electrophoresed on native 4% acrylamide gels (37.5:1 ratio of acrylamide to bisacrylamide) containing 2.5% glycerol in 25 mM Tris base, 190 mM glycine, and 1 mM EDTA (pH 8.3). The gels were run at 13 V/cm in the cold room. Following electrophoresis, the gels were transferred to filter paper, dried under vacuum, and autoradiographed with intensifying screens at −70 °C.

RESULTS

Bacterially expressed MBP/EmBP-1 fusion protein bound labeled Em1a probe in a concentration-dependent manner (Fig. 1, *lanes 2* and *3*). Nuclear extracts prepared from mature wheat embryos also bound Em1a probe, forming complexes with mobilities different from that of MBP/EmBP-1 (data not shown). Nuclear extracts containing 450 ng of protein (or less) did not show any protein-DNA complex formation with labeled Em1a probe (Fig. 1, *lane 4*). However, when 450 ng of nuclear extract was added to DNA binding reactions containing MBP/EmBP-1, an 80-fold increase in DNA binding activity of MBP/EmBP-1 was observed (Fig. 1, compare *lanes 3* and *5*). We found no differences between nuclear extracts prepared from maize wild type and *vp1* mutant embryos in their ability to enhance the binding of EmBP-1 to Em1a (data not shown). We also observed no differences in enhancing activity between extracts prepared from ABA-treated or untreated embryogenic rice suspension cells (data not shown). From these results, we conclude that the
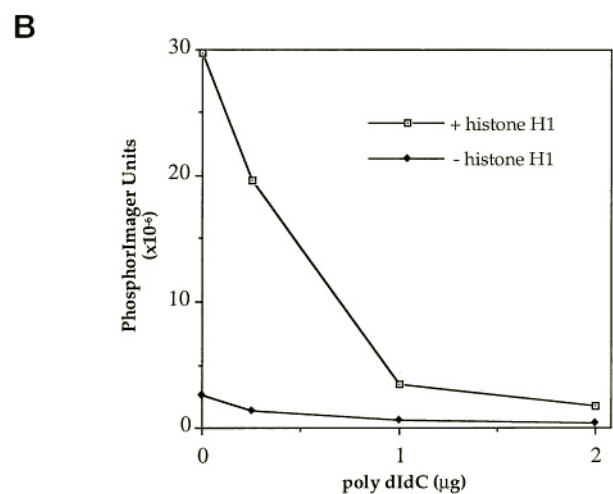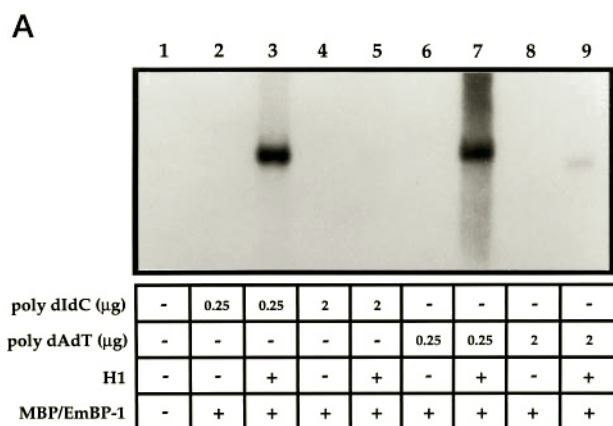

FIG. 2. **The trichloroacetic acid-soluble proteins histone H1 and HMGb enhance the DNA binding activity of MBP/EmBP-1.** Electrophoretic mobility shift assay was performed using Em1a as a probe. *Lanes*: *1*, free probe; *2*, 9 ng of MBP/EmBP-1; *3*, 100 ng of purified H1; *4*, 100 ng of H1 + 9 ng of MBP/EmBP-1; *5*, 100 ng of HMGb; *6*, 100 ng of HMGb + 9 ng of MBP/EmBP-1; *7*, 100 ng of HMGc/d; *8*, 100 ng of HMGc/d + 9 ng of MBP/EmBP-1; *9*, 500 ng of MBP; *10*, 500 ng of MBP+ 9 ng of MBP/EmBP-1.

enhancement activity observed in nuclear extracts is neither altered by ABA treatment nor can it be attributable solely to the presence of the maize transcriptional activator protein, VP1.

In an effort to identify the proteins that are involved in this enhancement activity, we prepared a 2% trichloroacetic acid-soluble fraction from wheat embryo nuclear extracts and tested its ability to enhance DNA binding activity. The trichloroacetic acid-soluble protein fraction did not bind Em1a probe alone (Fig. 1, *lane 6*) but clearly demonstrated enhancing activity (Fig. 1, compare *lanes 3* and *7*). HMG and histone H1 proteins are present in nuclear extracts at high levels and are soluble in trichloroacetic acid (Ref. 9 and data not shown).

We tested the abilities of highly purified histone H1 and HMG proteins to enhance the DNA binding activity of MBP/EmBP-1. Purified H1 protein (100 ng) stimulated the DNA binding activity of MBP/EmBP-1 up to 80-fold (Fig. 2, compare *lanes 2* and *4*) when added to a standard DNA binding reaction. Under these conditions, histone H1 alone does not bind the probe (Fig. 2, *lane 3*). When used at similar concentrations, HMGb also enhanced MBP/EmBP-1 binding (Fig. 2, *lane 6*). However, addition of a mixture of HMGc and -d at a similar concentration, or purified MBP, did not result in an enhancement (Fig. 2, *lanes 8* and *10*).

In order to determine if histone H1 was interacting with DNA nonspecifically, we titrated the amount of nonspecific competitor DNA in the binding reactions and determined its effect on enhancement. Fig. 3*A* shows that enhancement of DNA binding activity by H1 was dependent on the concentrations of poly(dI·dC) or poly(dA·dT), two nonspecific DNA competitors, present in our binding reactions. In the presence of 0.25 μg of poly(dI·dC), histone H1 did not shift the Em1a probe (Fig. 3*A*, *lane 2*). However, when histone H1 is added to MBP/EmBP-1, DNA binding activity was stimulated (Fig. 3*A*, *lane 3*). If the amount of poly(dI·dC) in the DNA binding reaction was increased to 2 μg, the ability of H1 to enhance DNA binding was significantly reduced (Fig. 3*A*, *lane 5*). Poly(dI·dC) had only a marginal effect on the binding activity of MBP/EmBP-1 in the absence of H1, while the enhancement effect caused by H1 was eliminated at high concentrations (2 μg) of this nonspecific competitor (Fig. 3*B*). At the same concentrations, poly(dA·dT) was equivalent to poly(dI·dC) at competing the enhancement effect (Fig. 3*A*, *lanes 2–5 versus lanes 6–9*). In the absence of nonspecific competitor DNA (poly(dI·dC)), as little as 1 ng of purified H1 protein still caused the enhancement effect (Fig. 4). At this concentration of H1, there was approximately a 1:1 stoichiometry of H1 to the Em1a probe in the DNA binding reactions. From these results, we conclude

A

	1	2	3	4	5	6	7	8	9
poly dIdC (µg)	-	0.25	0.25	2	2	-	-	-	-
poly dAdT (µg)	-	-	-	-	-	0.25	0.25	2	2
H1	-	-	+	-	+	-	+	-	+
MBP/EmBP-1	-	+	+	+	+	+	+	+	+

B

FIG. 3. **Enhancement activity is dependent on the concentration of nonspecific competitor DNA.** *A*, electrophoretic mobility shift assay using Em1a as a probe. *Lane 1*, free probe. *Lanes 2–9* each have 9 ng of MBP/EmBP-1 fusion protein. *Lanes 3, 5, 7,* and *9* each contain 100 ng of histone H1. *Lanes 2* and *3* have 250 ng of poly(dI·dC), *lanes 4* and *5* have 2 µg of poly(dI·dC), *lanes 6* and *7* have 250 ng of poly(dA·dT), and *lanes 8* and *9* have 2 µg of poly(dA·dT). *B*, electrophoretic mobility shift assay was performed using Em1a as a probe, with varying amounts (µg) of poly(dI·dC) in the presence or absence of histone H1 (100 ng). The amount of shifted Em1a probe was quantitated on a Molecular Dynamics PhosphorImager and plotted.

that histone H1 enhances DNA binding activity by interacting nonspecifically with the DNA.

DISCUSSION

Nuclear extracts isolated from embryogenic rice suspension cells treated with ABA have an increased amount of Region I binding activity compared with nuclear extracts from untreated cells (6). Mutations in the Em1a element within Region I not only reduced ABA-induced expression in a transient assay but prevented binding by the bZIP factor EmBP-1 (6). We observed in this study that nuclear extracts from wheat and maize embryos enhanced the DNA binding activity of recombinant EmBP-1 to the ABA-response element in Region I of the *Em* promoter, *i.e.* Em1a. At the concentrations used, these nuclear extracts showed no DNA binding activity to Em1a as measured by electrophoretic mobility shift assay. However, in DNA binding reactions, these extracts were able to enhance (up to 80-fold) the binding of recombinant EmBP-1 to Em1a. Addition of the maize transcriptional activator VP1 to DNA binding reactions, containing various recombinant transcription factors and their target sites (including EmBP-1 and Em1a), resulted in a similar enhancement (7). However, we have shown in this study that the enhancement activity of nuclear extracts was

MBP/EmBP-1:	+	-	+	-	+	-	+
Histone H1:		5ng		2ng		1ng	
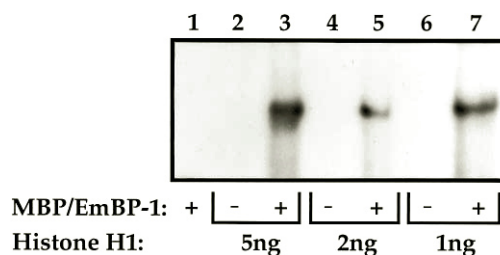
FIG. 4. **Enhancement of MBP/EmBP-1 DNA binding activity in the absence of poly(dI·dC).** Electrophoretic mobility shift assay using Em1a as a probe, in the absence of poly(dI·dC). *Lanes*: *1*, 2 ng of MBP/EmBP-1; *2*, 5 ng of histone H1; *3*, 5 ng of H1 + 2 ng of MBP/EmBP-1; *4*, 2 ng of H1; *5*, 2 ng of H1 + 2 ng of MBP/EmBP-1; *6*, 1 ng of H1; *7*, 1 ng of H1 + 2 ng of MBP/EmBP-1.

not due solely to VP1, since nuclear extracts from *vp1* mutant and wild type maize embryos equally enhanced DNA binding activity of EmBP-1.

To further characterize this enhancing activity, we showed that a trichloroacetic acid-soluble fraction from wheat embryo nuclear extracts retained the ability to stimulate DNA binding activity. This fraction from wheat embryo extracts is enriched for four HMG proteins (a, b, c, and d) and histone H1 (Ref. 9 and data not shown). We obtained highly purified H1 and HMG proteins and tested their abilities to enhance the DNA binding activity of EmBP-1. Both H1 and HMGb proteins enhanced the DNA binding activity of EmBP-1, while a mixture of HMGc and HMGd at a similar concentration had no effect. Hence, at least these two factors can enhance the ability of EmBP-1 to bind to Em1a and account for at least a part of the total enhancement activity found in nuclear extracts. Purified HMG-1 protein from mammalian cells has been demonstrated to enhance the DNA binding activity of progesterone receptors (10). Our data confirm this result since HMGb from wheat is equivalent to HMG-1 from mammalian cells (11). However, the enhancement of a specific DNA binding protein by histone H1 as reported in this study appears to represent a novel activity for H1.

In order to determine if H1 was interacting with DNA, we altered the amount of nonspecific competitor DNA in our binding reactions. We have shown that the enhancement effect is eliminated with increasing amounts of poly(dI·dC) in the binding reactions, indicating that the H1 is likely binding to the poly(dI·dC). Since histone H1 has been shown to bind to AT-rich tracts of DNA (12), we compared the abilities of poly(dI·dC) and poly(dA·dT) to compete for enhancement. Poly(dA·dT) and poly(dI·dC) were equal in their ability to compete for enhancement, indicating that H1 interacts with each equally well under our binding conditions. Addition of up to 1 µg of polylysine to DNA binding reactions had no effect on EmBP-1 DNA binding activity (7). This indicates that H1 is 100 times more efficient at enhancement than addition of positively charged peptides to DNA binding reactions. In the absence of nonspecific competitor DNA (poly(dI·dC)), we have shown that as little as 1 ng of H1 still enhanced the DNA binding activity of EmBP-1. At 1 ng of H1, there is approximately a 1:1 ratio of H1 to DNA in our binding reactions. Hence, under these conditions we have drastically altered the ratio of H1 to EmBP-1 with no effect on enhancement. This result suggests that H1-induced enhancement probably occurs through H1 interactions with the DNA element Em1a rather than with EmBP-1.

Several reports have appeared in the literature describing the phenomena of accessory factors in nuclear extracts enhancing the DNA binding activity of a number of transcription factors (13–19). In several of these reports, the authors simply described an enhancing activity present in nuclear extracts but did not identify specific factors that were responsible for the

observed effects. However, a protein (REF-1) has been characterized and shown to mediate enhancement of AP-1 DNA binding activity by modulating the redox potential in the DNA binding reactions (20). REF-1-enhancing activity was mimicked by the addition of high concentrations of dithiothreitol to the DNA binding reactions. In our studies, dithiothreitol had no effect on EmBP-1 binding activity (data not shown).

How might plant proteins such as histone H1 and HMGb cause an enhancement of DNA binding activity as we have described in this study? In the case of steroid receptors, HMG-1 interacts with DNA and increases DNA flexibility, resulting in enhanced binding by the progesterone receptor (10). The maize HMGa protein, a homolog of wheat HMGb, also increases DNA flexibility (21). This may be the mechanism responsible for our observations, since we have shown that highly purified histone H1 and HMGb proteins from wheat are able to enhance the DNA binding activity of EmBP-1 through their interaction with DNA. It has been proposed that proteins with a high concentration of positive charges on their surface interact with DNA and induce conformational changes in the DNA by neutralizing the negative charges along the phosphodiester backbone (22). This theoretical model has been tested by replacing the negatively charged phosphates along one side of a DNA strand with neutral methylphosphonate groups (23). Neutralizing the negative charges along one side of a DNA molecule induces a bend in the DNA. These observations support the hypothesis that positively charged proteins could interact with DNA and neutralize the phosphodiester backbone in a similar manner, thus inducing a bend in the DNA. According to this model, any protein with a high concentration of positive charges at its surface should interact with DNA, induce a bend, and enhance DNA binding. Wheat histone H1 is a highly positively charged protein (pI 11.6) and could be inducing a bend in Em1a, thus facilitating binding by EmBP-1.

Enhancement of the DNA binding activities of specific nuclear proteins, triggered by ABA, could result in an increased rate of transcription from the ABA-regulated *Em* promoter. The goal of this study was to identify factors in nuclear extracts that enhance the binding activity of proteins that recognize ABA and VP1-responsive elements in the *Em* promoter. We identified histone H1 and HMGb as factors in nuclear extracts that can enhance binding of EmBP-1 to Em1a. Our results, which indicate for the first time that histone H1 possesses this activity, lend further support to the model that positively charged proteins can drastically affect the DNA binding activity of specific transcription factors. Furthermore, our study points to these chromosomal proteins as potential targets of an ABA-mediated modification, *e.g.* acetylation (24), that could affect the regulation of *Em* gene expression.

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