Three *Arabidopsis* MBF1 Homologs with Distinct Expression Profiles Play Roles as Transcriptional Co-activators

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Short Communication

Multiprotein bridging factor 1 (MBF1) is known to be a transcriptional co-activator that mediates transcriptional activation by bridging between an activator and a TATA-box binding protein (TBP). We demonstrated that expression of every three MBF1 from *Arabidopsis* partially rescues the yeast *mbf1* mutant phenotype, indicating that all of them function as co-activators for GCN4-dependent transcriptional activation. We also report that each of their subtypes shows distinct tissue-specific expression patterns and responses to phytohormones. These observations suggest that even though they share a similar biochemical function, each MBF1 has distinct roles in various tissues and conditions.

Keywords: ABA — *Arabidopsis thaliana* — bZIP — co-activator — MBF1 — transcription.

Abbreviations: 3-AT, 3-aminotriazole; ABRE, ABA-response element; bZIP, basic region/leucine zipper; EMSA, electrophoresis mobility shift assay; GST, glutathione S-transferase; MBF1, multiprotein bridging factor 1; ORF, open reading frame; SA, salicylic acid; TBP, TATA-box binding protein.

Transcriptional regulation plays a major role in expression of the genomic information during complex biological processes. The effect of binding of transcription factors to cis-elements must be transmitted to RNA polymerase to ensure transcription initiation and maintain active transcription. In humans and yeast, it has been reported that transcriptional co-activators activate gene expression by connecting a transcription factor with components of the basal transcriptional machinery (Kwok et al. 1994, Ge and Roeder 1994, Knaus et al. 1996). However, the function of co-activators from plants has been poorly understood.

One co-activator, multiprotein bridging factor 1 (MBF1), was first purified from posterior silk gland extracts of the silkworm, *Bombyx mori*. MBF1s from insects stimulate transcription from the *fushi tarazu* promoter in vitro through its binding to the TATA-box binding protein (TBP) and a nuclear receptor FTZ-F1 (Li et al. 1994, Takemaru et al. 1997). MBF1 also mediates transcriptional activation by bridging between a basic region/leucine zipper (bZIP)-type transcriptional activator and TBP in yeast (Takemaru et al. 1998), human (Kabe et al. 1999) and *Drosophila* (Liu et al. 2003). In plants, it has been shown that *ER24* gene, a tomato counterpart of MBF1 (LeMBF1), is immediately and transiently induced in ethylene-treated late immature fruit (Zegzouti et al. 1999). Transcription of potato *MBF1 (SIMBF1)* is also up-regulated during fungal attack and upon wounding (Godoy et al. 2001). Furthermore, it has been reported that phosphorylation of StMBF1 is promoted by the treatment with hyphal cell wall components derived from *Phytophthora infestans* both in vitro and in vivo (Zanetti et al. 2003). Tobacco MBF1 (NtMBF1a) and two *Arabidopsis* MBF1s (AtMBF1a and AtMBF1b) have been shown to interact with the tomato mosaic virus movement protein (Matsushita et al. 2002). These observations suggest that, so far, all plant MBF1 homologs reported are involved in defense responses to pathogen.

In this study we tested whether MBF1s from *Arabidopsis thaliana* are functional for complementation of a yeast mutant lacking MBF1 function. We did the test because the function of plant MBF1 as a multiprotein bridging factor has never been demonstrated. We found three candidates of MBF1 homologs (AtMBF1a, AtMBF1b, and AtMBF1c) in a public database of the *A. thaliana* genome whose deduced amino acid sequences had considerlable identitides to those of MBF1s from other eukaryotes. So far existence of two subtypes of MBF1 has been reported only in humans. The two proteins share identical amino terminal regions (129 aa), but differ slightly in their carboxyl-terminal sequences (Kabe et al. 1999). Only one gene for MBF1 has been reported in all other eukaryotes. *A. thaliana*, which has three subtypes of MBF1 genes, is the first such example among all organisms. It is interesting whether each *MBF1* gene has a distinct biological role. This study is also intended to reveal a tissue-specific expression pattern of each subtype of MBF1s in *A. thaliana*. We also tested effects of

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addition of phytohormones and precursor on expression of each gene in vivo.

It has been shown that amino acid sequences of MBF1 proteins are widely conserved among eukaryotes (Takemaru et al. 1997). To characterize three subtypes of MBF1s from Arabidopsis, the deduced amino acid sequences were compared with those of MBF1s from other eukaryotes (Fig. 1A) and a phylogenic tree was constructed with full-length amino acid sequences (Fig. 1B). All MBF1s are broadly classified into four groups (groups 1–4); plant MBF1s are classified into two groups (groups 2 and 3) among these four (Fig. 1B). The extent of amino acid sequence identity between AtMBF1a and AtMBF1b is 92%; these MBF1s belong to the same group (group 2) as StMBF1 reported by Godoy et al. (2001). Amino acid sequence identities of StMBF1 with AtMBF1a and AtMBF1b are 81% and 83%, respectively. AtMBF1c resides in the group 3 and shares 67% amino acid identities with LeMBF1 reported by Zegzouti et al. (1999), while it shows a
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![Fig. 2](https://academic.oup.com/pcp/article-abstract/45/2/225/1814956)

(A) Binding of GCN4 and yTBP to AtMBF1s. Binding of bacterially expressed and affinity-purified His-GCN4 and His-yTBP to GST-AtMBF1s were analyzed by far-Western analysis. (A and B) Equal amounts (2 µg) of affinity-purified GST-AtMBF1s and GST were separated on 12% polyacrylamide gel containing SDS and transferred onto a nitrocellulose membrane (Hybond-ECL; Amersham Biosciences) using a semi dry blotter (ATTO Bioscience, Tokyo, Japan). The blotted membrane (20 cm²) was incubated in TBST (20 mM Tris-HCl pH 7.5, 150 mM NaCl, and 0.1% Tween-20) containing 5% skim milk at room temperature for 1 h and then incubated in reaction mixture (3 ml) containing 20 mM HEPES-KOH (pH 8.0), 6 mM MgCl₂, 60 mM KCl, 1 mM dithiothreitol, and 0.1% Nonidet P-40 in the presence of 30 µg of His-yTBP at 4°C for 8 h (A) or in the presence of 300 µg of His-GCN4 at 4°C for 16 h (B). The membrane was washed three times for 30 min in the same reaction mixture in the absence of protein at room temperature, and then His fusion proteins bound to AtMBF1 were fixed tightly for 25 min onto the membrane by capillary action driven by laminated paper towel fixed at the back side of the membrane in a blotting buffer containing 100 mM Tris-base, 192 mM glycine, and 20% methanol. The surface of the original membrane was protected by wrapping in one sheet of a new nitrocellulose membrane (Hybond-ECL) to prevent diffusion of interacting proteins from the original membrane. The original membrane was incubated in TBST containing 5% skim milk for 1 h at room temperature, and then His-yTBP and His-GCN4 bound to AtMBF1s were recognized by anti-His-Tag Monoclonal antibody (Novagen). The membrane was incubated with substrate for alkaline phosphatase (ECF substrate; Amersham Biosciences) after further incubation with a second antibody, goat anti-mouse IgG alkaline phosphatase conjugate (Novagen), and His-yTBP and His-GCN4 were visualized by detecting chemiluminescence using fluorImager 595 (Molecular Dynamics, Sunnyvale, CA, U.S.A.). (C) Equal amount (2 µg) of affinity-purified GST-AtMBF1s and GST were separated on 12% polyacrylamide gel containing SDS and stained by Coomassie brilliant blue.

Fig. 2A shows direct bindings of all purified GST-AtMBF1 fusion proteins to purified recombinant yTBP. Fig. 2B shows direct bindings of all purified GST-AtMBF1 fusion proteins to purified recombinant GCN4. GST polypeptide itself did not show any interaction to yTBP and GCN4 in both analyses as negative control.

We also analyzed interaction between AtMBF1s and yeast GCN4 by electrophoresis mobility shift assay (EMSA). A significant elevation of DNA-binding activity of GCN4 to its target DNA sequence was observed when each purified recombinant AtMBF1 was added to the binding reaction mixtures (Fig. 3, lanes 1 and 2–4). But we have never detected any band corresponding to a ternary complex containing AtMBF1, GCN4, and its target DNA. Although we have detected two faint bands indicated by asterisks in Fig. 3, appearances of these bands were not dependent on AtMBF1 addition to the reaction mixture (data not shown). It has been reported that no interaction of GCN4 with yeast MBF1 occurred in the absence of Mg²⁺ and the complex that formed in the presence of Mg²⁺ dissociated immediately upon removal of Mg²⁺ (Takemaru et al. 1998). Therefore the ternary complex including AtMBF1 cannot be expected to be observed because of immediate dissociation of the GCN4-AtMBF1 complex in the electrophoresis buffer without Mg²⁺.

Fig. 3. Binding of MBF1s to yeast GCN4 and yeast TBP (yTBP). We employed far-Western analysis. Fig. 2A shows direct bindings of all purified glutathione S-transferase (GST)-AtMBF1 fusion proteins to purified recombinant yTBP. Fig. 2B shows direct bindings of all purified GST-AtMBF1 fusion proteins to purified recombinant GCN4. GST polypeptide itself did not show any interaction to yTBP and GCN4 in both analyses as negative control.

It has been described that the yeast MBF1 (yMBF1) mediates the GCN4-dependent transcriptional activation of the HIS3 gene by binding between GCN4 and TBP. Each yeast mutant lacking either MBF1 or GCN4 is reported to be viable, but sensitive to 3-aminotriazole (3-AT), an inhibitor of the 3AT strain (KT130; Takemaru et al. 1998) and the mbf1 strain, KT131; Takemaru et al. 1998) in the presence of 3-AT in the histidine-free medium, which contains 1% galactose and 1% raffinose as carbon sources. While the ∆mbf1 strain transformed with an empty vector, pYES2, (Invitrogen, Carlsbad, CA, U.S.A.) was sensitive to 3-AT, WT strain (KT130; Takemaru et al. 1998) and the ∆mbf1 strains transformed with plasmid containing yeast MBF1 (pyMBF1) grew on a histidine-free medium in the presence of 3-AT (Fig. 1D). Expression of every AtMBF1 cDNA partially recovered the growth of the ∆mbf1 strain upon histidine starvation (Fig. 1D) as in the case of Drosophila MBF1 (Liu et al. 2003), indicating that three AtMBF1s all play a role of co-activator for GCN4-dependent transcriptional activation in place of the yeast MBF1 in yeast cells. All yeast strains used in this experiment showed essentially the same normal growth rate on a histidine-free medium in the absence of 3-AT (Fig. 1C).

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We observed no significant difference in the effect of enhancement of DNA binding activity among three types of AtMBF1. Specificity of GCN4 binding to the labeled oligo DNA was confirmed by observing a reduction of GCN4-DNA complex formation by the addition of unlabeled oligo DNA as a competitor (data not shown). Similar elevation of bZIP type transcription factor binding to DNA fragment has
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been reported when yMBF1 (Takemaru et al. 1998) or hMBF1 (Kabe et al. 1999) was added to the binding reaction mixtures of EMSA. These observations suggest that contacts of AtMBF1s with the DNA-binding domain of GCN4 induce a conformational change in GCN4, allowing increased binding to its target DNA sequence. A database search by Jakoby et al. (2002) has revealed 75 distinct members of the bZIP family in Arabidopsis genome. Positions of four arginine residues in the basic region of the yeast GCN4 that are considered to be necessary for its binding to yMBF1 (Takemaru et al. 1998), are all conserved in the corresponding regions of some of the bZIP proteins from A. thaliana, including OBF4/TGA4, an ocs element binding protein (Buttner and Singh 1997, Zhang et al. 1993, Zhang et al. 1995) and ABI5, a factor involved in ABA signal transduction pathway (Carles et al. 2002, Lopez-Molina et al. 2002, Finkelstein and Lynch 2000). These transcription factors will be possible candidates for partner of AtMBF1s in Arabidopsis.

The mRNA expression pattern of each subtype of AtMBF1 was also investigated by Northern blot analysis using total RNA from different tissues of Arabidopsis plants (4–6 weeks old) or whole seedlings (8 d old). As shown in Fig. 4, we observed a similar expression pattern for AtMBF1a and AtMBF1b. While these two genes show relatively higher expression in flower, AtMBF1c shows lower expression in the same tissue. Expression of all AtMBF1 mRNAs was observed in almost all mature plant tissues. These observations suggest that similar promoters control expression of both AtMBF1a and AtMBF1b. However, the expression of AtMBF1c gene is controlled in a totally different manner from other two genes.

We tested whether mRNA levels of each AtMBF1 were affected by ethylene, salicylic acid (SA), or other phytohormones because it has been reported that the expression of MBF1 from tomato (Zegzouti et al. 1999) or potato (Godoy et al. 2001) are stimulated by ethylene or its precursor and SA, respectively. Ten-day-old seedlings were transferred into a liquid medium each containing phytohormones or precursor; then, mRNA accumulation of each AtMBF1 was determined by Northern blot analysis. As a control experiment, change in mRNA level of each gene after 24 h was investigated. mRNA level of AtMBF1c significantly increased in the presence of 0.1% ethanol (solvent for phytohormones) alone, while such prominent change was not observed in mRNA levels of AtMBF1a and AtMBF1b (Fig. 5, Control). This accumulation of AtMBF1c mRNA was also observed even in the absence of ethanol (data not shown). Since seedlings should be soaked...
increase is thought to be caused by physical stress or anaerobic stress. Several GT-motifs (AAACCA), which are known to be necessary for low oxygen induction of the alcohol dehydrogenase gene (ADH1; Dolferus et al. 1994, Hoeren et al. 1998), were found in the promoter of the LeMBF1 gene. Although no difference in the effect of GCN4 binding to DNA was observed, significant differences at the amino acid level between group 2 (containing AtMBF1a and AtMBF1b) and group 3 (containing AtMBF1c) imply that these AtMBF1s may have distinct interacting partners in A. thaliana. Studies on characterization of AtMBF1s binding to AtTBP and finding of a real partner of AtMBF1s in A. thaliana are in progress.

The Columbia ecotype of A. thaliana (L.) Heynh. was used for all experiments in this report. Seeds were sterilized with 1.5% (v/v) sodium hypochlorite and 0.02% Triton X-100 for 3 min with vigorous shaking, then washed five times with sterile water, and plated on half-strength MS medium (Murashige and Skoog 1962) solidified with 0.8% phytoagar (Wako Pure Chemical Inds., Osaka, Japan) and supplemented with half-strength Gamborg B5 vitamins (Gamborg et al. 1968) and 1% sucrose. After the plates were placed at 4°C for 3 d in the dark to break residual dormancy, plants were grown under continuous white light at 22°C. Seeds were sown on 1 : 1 (v/v) mixture of vermiculite and Metromix 250 (Hyponex, Marysville, OH, U.S.A.) irrigated with 0.1% Hyponex (Hyponex) in a pot and grown under continuous light at 22°C for analysis of tissue-specific expression patterns. Seedlings were grown on MS solid medium for 10 d for treatments with phytohormones and precursor.

Total RNA was isolated using TRIZOL® Reagent as described by the manufacturer (Invitrogen). For Northern blot analysis, total RNA was separated on a 1.2% agarose gel containing 2.2 M formaldehyde, then transferred to Hybond-N+ nylon membranes (Amersham Biosciences, Piscataway, NJ, U.S.A.). The RNA on membranes were hybridized with 32P-labeled DNA probes in 10 mM NaHPO4 (pH 7.2), 7% (w/v) SDS, and 1 mM EDTA at 60°C for 16 h. Each 32P-labeled gene specific DNA probe was produced from each corresponding PCR product using the Megaprime DNA Labelling System (Amersham Biosciences). Sequences of specific primers used for preparation of specific probes were: 5′-ACTGATGTTAGCA-AGTAAACAGAATC-3′ and 5′-CACAATGTTAGGAAAAG-ACCCAGC-3′ for AtMBF1a probe, 5′-AAATGAAGACCA-
AGCTCTTAAAGG-3' and 5'-ATAATGACAAAAAGGTCCA-AACAGC-3' for AtMBF1b, 5'-TGTTCTTTTCTCTCATTC-ATCGAC-3' and 5'-CATTTATCAAACAAAACCAAGAC-3' for AtMBF1c. Membranes were washed once in 1× SSC containing 0.1% (w/v) SDS at room temperature for 10 min, then washed three times in 0.5× SSC containing 0.1% (w/v) SDS at 68°C for 10 min.

The three AtMBF1 and yMBF1 cDNAs were isolated from 4-week-old Arabidopsis plants and yeast WT strain KT130 by RT-PCR, then cDNA fragments were ligated into pGEM-T Vector (Promega, Madison, WI, U.S.A.), and their nucleotide sequences were analyzed. For preparing plasmid DNAs used in yeast transformation for yeast complementation test, the four sequences were analyzed. For preparing plasmid DNAs used in RT-PCR, then cDNA fragments were ligated into pGEM-T vector (Amersham Biosciences). Proteins were purified by Glutathione Sepharose ® 4B (Amersham Biosciences) chromatography. ORF of AtMBF1b was overproduced in E. coli strain BL21(DE3)pLysS as inclusion body and further purified using Ni-affinity column (His-bind resin, Novagen). Histidine- 

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References


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