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Mutual Regulation of *Arabidopsis thaliana Ethylene-responsive Element Binding Protein* and a Plant Floral Homeotic Gene, *APETALA2*

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• *Background and Aims* It has previously been shown that *Arabidopsis thaliana ethylene-responsive element binding protein* (*AtEBP*) contributed to resistance to abiotic stresses. Interestingly, it has also been reported that expression of *ethylene-responsive factor* (*ERF*) genes including *AtEBP* were regulated by the activity of APETALA2 (AP2), a floral homeotic factor. *AP2 is* known to regulate expression of several floral-specific homeotic genes such as *AGAMOUS*. The aim of this study was to clarify the relationship between *AP2* and *AtEBP* in gene expression.
• *Methods* Northern blot analysis was performed on *ap2* mutants, ethylene-related *Arabidopsis* mutants and transgenic *Arabidopsis* plants over-expressing *AtEBP*, and a T-DNA insertional mutant of *AtEBP*. Phenotypic analysis of these plants was performed.
• *Key Results* Expression levels of *ERF* genes such as *AtEBP* and *AtERF1* were increased in *ap2* mutants. Over-expression of *AtEBP* caused upregulation of *AP2* expression in leaves. *AP2* expression was suppressed by the null-function of ethylene-insensitive2 (EIN2), although *AP2* expression was not affected by ethylene treatment. Loss of AtEBP function slightly reduced the average number of stamens.
• *Conclusions AP2 and AtEBP* are mutually regulated in terms of gene expression. *AP2* expression was affected by EIN2 but was not regulated by ethylene treatment.

Key words: *APETALA2*, *Arabidopsis thaliana*, *AtEBP*, *ERF*, EIN2, EIN3.

INTRODUCTION

Co-ordinated regulation of gene expression is an essential biological event, especially when each transcriptional factor acts as a key regulator. In *Arabidopsis*, the floral meristem produces four concentric whorls of floral organs (sepals, petals, stamens and carpels). According to the 'ABC' model for the determination of floral organ identity, A activity specifies sepals, A and B activities lead to petals, B and C activities lead to stamens, and C activity specifies carpels (Weigel and Meyerowitz, 1994).

The *APETALA2* (*AP2*) gene, which belongs to the A class of genes, exhibits several characteristics distinct from other ABC genes. Although most ABC genes contain a MADS domain, *AP2* contains two APETALA2/ethylene-responsive element binding protein (AP2/EREBP) domains (Jofuku *et al.*, 1994). The *AP2* transcript is not observed in a region-specific pattern in the four wholes of flower, and is detected in other vegetative tissues (Jofuku *et al.*, 1994; Okamuro *et al.*, 1997). Recent reports showed that AP2 controlled seed mass (Jofuku *et al.*, 1994, 2005; Ohto *et al.*, 2005) and that expression of the AP2 protein was translationally regulated by the microRNA mi172 (Aukerman and Sakai, 2003; Chen, 2004). Thus, AP2 may play an important role in both floral and whole-plant development.

AP2 belongs to the AP2/EREBP family, one of the largest groups of plant transcriptional factors (Riechmann *et al.*, 2000). It is known that AP2 suppresses expression of *AGAMOUS*, the C gene of a floral homeotic gene (Drews *et al.*, 1991; Bomblies *et al.*, 1999). In addition, AP2 regulates the expression of *ethylene-responsive factor* (*ERF*) genes containing one AP2/EREBP domain (Okamuro *et al.*, 1997). However, the relationship of the transcriptional regulation between *AP2* and *ERF* genes is not fully understood.

Previously, we characterized *Arabidopsis thaliana ethylene-responsive element binding protein* (*AtEBP*), one of the *ERF* genes, as a transcriptional activator (Ogawa *et al.*, 2005). *AtEBP* is regulated by an ethylene signal (Büttner and Singh, 1997; Ogawa *et al.*, 2005). It was clarified that *AtEBP* is regulated by EIN2, but not EIN3, suggesting that *AtEBP* expression is independently regulated under EIN3 in ethylene signalling. Interestingly, it was reported that *AtEBP* expression was regulated by AP2 (Okamuro *et al.*, 1997). Nevertheless, relationships between *AtEBP* and *AP2* in ethylene signal transduction have not been investigated in detail.

Here, we show that AP2 regulates *ERF* genes such as *AtEBP* and *AtERF1*, and the over-expression of *AtEBP* causes the accumulation of *AP2* transcripts. The regulation of *AP2* in ethylene signalling and the functional role of AtEBP in floral development are also demonstrated.

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MATERIALS AND METHODS

Plant materials

The 'Columbia' ecotype of *Arabidopsis thaliana* was used. All plants were cultivated in growth chambers at 23 °C under continuous light. Ethylene-related mutants, *etr1-1*, *ein2-1*, *ein3-1* and *ctr1-1*, and a floral homeotic mutant, *ap2-5*, were obtained from the *Arabidopsis* Biological Resource Center (Columbus, OH, USA). Transgenic *Arabidopsis* plants over-expressing *AtEBP* were obtained as described previously (Ogawa *et al.*, 2005).

A knockout plant of *AtEBP* with a T-DNA insert was obtained from the Torrey Mesa Research Institute, USA. The knockout plants were selected on Murashige–Skoog medium containing 2·4 μg mL^{-1} glufosinate ammonium. The T3 generation of the homozygous plants confirmed that T-DNA was inserted in the ORF of *AtEBP* by use of genomic PCR amplification analysis.

Northern blot analysis

Plant tissues were homogenized with liquid nitrogen in the extraction buffer [200 mM Tris–HCl (pH 8·0), 10 mM ethylenediaminetetraacetic acid, 100 mM NaCl, 0·1 % SDS and 0·1 % mercapthoethanol]. Total RNAs (10 μg) were fractionated on 1·2 % agarose gel containing 5 % formaldehyde, and transferred to a nylon membrane (Biodyne B, Pall, Washington, NY). With regard to the ^{32}P-labelled probes, the 3′-untranslated region was used for *AtERF1* (Fujimoto *et al.*, 2000) and the C-terminals of the coding region, except the AP2/EREBP domain which was used for *AP2* and *AtEBP* (Jofuku *et al.*, 1994; Büttner and Singh, 1997).

Hybridization was performed in 10 % dextran sulfate solution containing 1M NaCl, 1 % SDS and 10 μg mL^{-1} heat-denatured salmon sperm DNA at 65 °C for overnight. Washing was performed with 2× SSC for 10 min, with 1× SSC containing 0·1 % SDS at 65 °C for 30 min and 0·1× SSC containing 0·1 % SDS at 65 °C for 30 min. The membranes were analysed using a BAS1500 imaging plate scanner (Fuji Film, Tokyo, Japan).

RESULTS

Effects of AP2 *on AtEBP expression*

It has been reported that expression of the *ERF* genes including *AtEBP* is regulated by AP2 activity (Okamuro *et al.*, 1997). This observation lead to the investigation of the transcriptional regulation of AP2/EREBP domain-containing genes in the current study. To investigate the relationships in the transcriptional regulation of *AP2* and *AtEBP*, an analysis was made of the expression patterns of *AP2* and *ERF* genes such as *AtEBP* and *AtERF1* in different tissues (flowers, stems and leaves). To avoid cross-hybridization among *AP2*, *AtEBP* and *AtERF1*, each specific probe was used for Northern blot hybridization (Fig. 1). In the wild type (WT), the *AtEBP* mRNA level was high in leaves and low in flowers, while *AP2* mRNA levels were low in all the tissues analysed (flowers, stems and leaves). There is a point-mutation in the AP2/EREBP domain of AP2 (residue Gly-159 to Glu) in the *ap2-5* mutant, which leads to reduced transcriptional activity of AP2 (Jofuku *et al.*, 1994). As a result, the floral homeotic phenotype was observed. To test whether AP2 activity affects the *AtEBP* expression, we investigated mRNA accumulation of *AtEBP* in *ap2-5*. In *ap2-5*, the *AtEBP* mRNA level was increased in flowers, leaves and stems. The *AP2* mRNA level of *ap2-5* was also increased, especially in flowers and leaves, suggesting that AP2 activity also suppresses its own *AP2* gene expression. The expression pattern of *AtERF1* was similar to that of *AtEBP*: the *AtERF1* mRNA level of *ap2-5* increased in flowers, leaves and stems compared with the WT. mRNA accumulation of these genes was examined in *ap2-7*, and similar results were obtained (data not shown). These

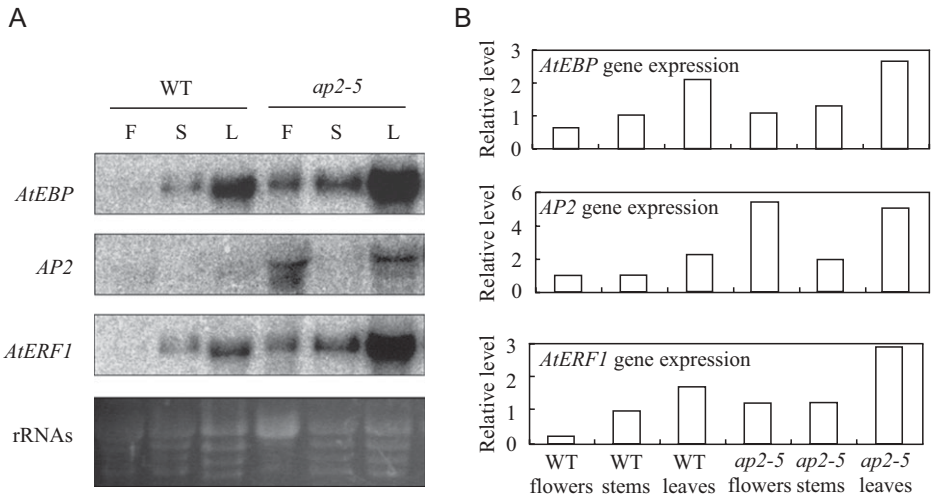

FIG. 1. Analysis of mRNA accumulation of *AtEBP*, *AP2* and *AtERF1* genes in WT and *ap2-5*. (A) Northern blot analysis. Total RNAs (10 μg) were isolated from flowers (F), stems (S) and rosette leaves (L) of 40-d-old plants. Ethidium bromide staining indicates rRNAs. (B) Relative levels of *AtEBP*, *AP2* and *AtERF1* expression. Each expression level was normalized to the rRNA bands, and the value for stems in WT was assigned as 1.

results may suggest that AP2 represses the expression of *ERF* genes such as *AtEBP*, *AtERF1* and the *AP2* gene itself.

Over-expression of AtEBP *and upregulation of AP2 expression*

In our previous work, it was demonstrated that AtEBP acts as a transcriptional activator (Ogawa *et al.*, 2005). In fact, over-expression of *AtEBP* in *Arabidopsis* resulted in the upregulation of plant defence genes such as *PDF1·2* and *GST6*. To determine the effects of AtEBP on the regulation of *AP2* expression, mRNA accumulation of *AP2* and *AtERF1* was investigated in leaves of *Arabidopsis* plants over-expressing *AtEBP*. As shown in Fig. 2, the *AP2* mRNA level was increased in *Arabidopsis* over-expressing *AtEBP*. The *AtERF1* mRNA level was also increased in these lines. These results suggest that AtEBP upregulates the expression of *AP2* and *AtERF1* genes directly or indirectly.

Analysis of the mRNA level of AP2 and other genes in ethylene mutants

AtEBP expression is regulated in the ethylene signalling pathway (Büttner and Singh, 1997; Ogawa *et al.*, 2005). Since over-expression of *AtEBP* caused upregulation of *AP2*, it was of interest to test whether *AP2* expression was also controlled through the ethylene signalling pathway. mRNA accumulation of *AP2* was investigated in ethylene-related *Arabidopsis* mutants: *ethylene resistant 1–1* (*etr1-1*), *ethylene insensitive 2-1* (*ein2-1*) and *ethylene insensitive 3–1* (*ein3-1*) mutants, which were isolated as ethylene-insensitive, and *constitutive triple response 1-1* (*ctr1-1*), which was isolated as a constitutive active mutant in the ethylene signalling pathway. The results showed that low-levels of *AP2* and *AtERF1* mRNAs were detected in *ein2-1* (Fig. 3); however, mRNA accumulation of these genes was not changed in *ctr1-1* compared with the WT.

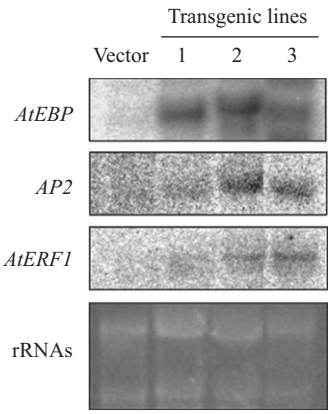

FIG. 2. *AP2* and *AtERF1* mRNA accumulations in transgenic *Arabidopsis* lines over-expressing *AtEBP*. Total RNAs (10 μg) were isolated from leaves of 35-d-old plants. Ethidium bromide staining indicates rRNAs.

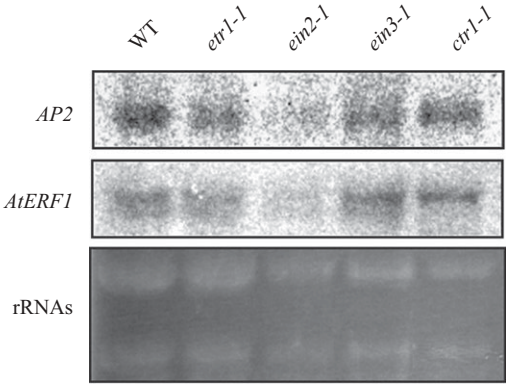

FIG. 3. Comparison of mRNA level of *AP2* and *AtERF1* in ethylene-related mutants. Total RNAs (10 μg) were isolated from leaves of 30-d-old plants. Ethidium bromide staining indicates rRNAs.

AP2 expression after ethephone treatment was also analysed (Fig. 4). *AtEBP* expression was increased in WT and the *ein3-1* mutant after ethephone treatment, suggesting that *AtEBP* expression was independent of the transcriptional control of EIN3. This result is consistent with our previous work (Ogawa *et al.*, 2005). In contrast, *ERF1* expression was increased in WT after ethephone treatment but not in *ein3-1*. It is known that *ERF1* expression is transcriptionally controlled by functional EIN3 (Solano *et al.*, 1998). *AP2* expression was not changed by ethephone treatment.

Floral phenotype in an AtEBP knockout plant

In order to understand the effect of AtEBP on plant development, a mutant line with a T-DNA insert in the *AtEBP* gene was analysed (Fig. 5A), and the *AtEBP* transcript was found to be lower in these plants (Fig. 5B). As shown in Fig. 6, the number of stamens in the *AtEBP* knockout plant was reduced compared with the WT. Five or four stamens were frequently observed in *AtEBP* knockout plants (approx. 20 % in 150 flowers in three

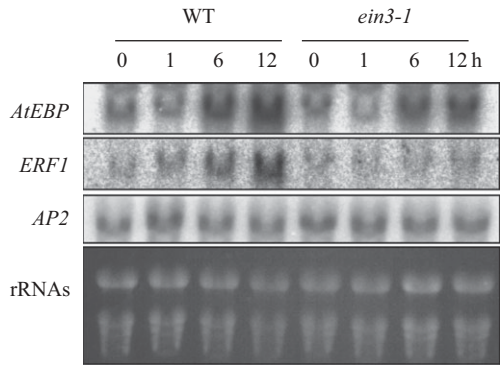

FIG. 4. Effects of ethylene on mRNA levels of *AtEBP*, *ERF1* and *AP2* in WT and *ein3-1* after ethylene treatment. Total RNAs (10 μg) were isolated from leaves of 30-d-old plants. The plants were sampled 0, 1, 6 and 12 h after spraying with 5 mM ethephone. Ethidium bromide staining indicates rRNAs.

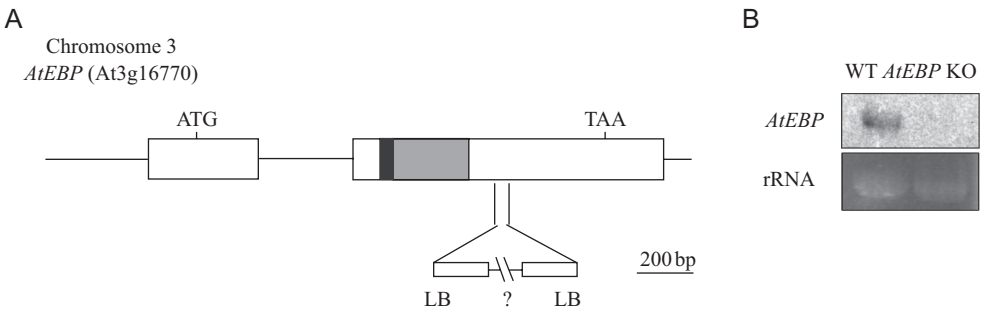

FIG. 5. The T-DNA insertional mutant of *AtEBP*. (A) Schematic diagram of the genomic *AtEBP* (At3g16770). White areas indicate the exon, black shading indicates the nuclear-located signal, and grey shading indicates the AP2/EREBP domain. LB indicates the light border of the T-DNA insertion. (B) Northern blot analysis of *AtEBP* knockout mutant plants. Total RNAs (10 μg) obtained from 30-d-old plants were loaded. The coding region of *AtEBP* was used as a probe. A gel stained with ethidium bromide is shown as a control.

independent experiments; Fig. 6E, F), although six stamens were observed in the WT and the vector control line (Fig. 6A, B). Such a phenotype was not observed in the *AtEBP* over-expression lines (Fig. 6C, D).

DISCUSSION

Recent studies have shown that AP2 plays a global role not only in floral development but also in the control of seed mass (Jofuku *et al.*, 1994, 2005; Okamuro *et al.*, 1997; Ohto *et al.*, 2005). In addition, *AP2* expression is controlled transcriptionally and translationally in a co-ordinated manner. In particular, micro RNAs are thought to target mRNAs of *AP2* and its homologs, thereby inhibiting the translation process (Aukerman and Sakai, 2003; Chen, 2004). However, the transcriptional regulation of AP2 has not been well understood (Okamuro *et al.*, 1997).

The current study showed that AP2 activity repressed *AtEBP*, *AtERF1* and *AP2* expression. This is consistent with previous results showing that AP2 regulates its own *AP2* expression (Okamuro *et al.*, 1997; Chen, 2004) as well as other genes, such as the *ERF* genes.

In addition, over-expression of *AtEBP* increased the expression level of *AP2*. AtEBP is a transcriptional activator interacting with GCC-box, an ethylene-responsive element (Büttner and Singh, 1997). Although the over-expression of *AtEBP* up-regulated *AP2* and *AtERF1* expression, these promoters (~2·0 kb upstream from ATG) did not contain the GCC-box. Interestingly, analysis of tomato ERF Pti4 interacting with GCC-box revealed that Pti4 bound to promoters in the absence of GCC-box (Chakravarthy *et al.*, 2003). Like Pti4, transcriptional regulation of the target genes of AtEBP may be complex.

Down-regulation of *AP2* was observed in *ein2-1*. The null mutation of EIN2 resulted in a complete loss of responsiveness to ethylene, suggesting that EIN2 is essential in the ethylene signal pathway. However, *AP2* expression was not induced by ethylene treatment or in *ctr1-1*, indicating that EIN2 is a receiver for various signals. It is known that EIN2 receives not only ethylene but also other signals, such as paraquat and jasmonic acid (Alonso *et al.*, 1999). The N-terminal of EIN2 is thought to be necessary for ethylene responsiveness. On the other hand, the C-terminal of EIN2 is required for transducing the signal to the downstream components (Wang *et al.*, 2002). Our observations suggested that the *AP2* expression was induced via EIN2 but not by the ethylene signal (Fig. 7).

The *AP2* mRNA level did not change in the *ein3-1* mutant. The position of EIN3 is a branch of the ethylene

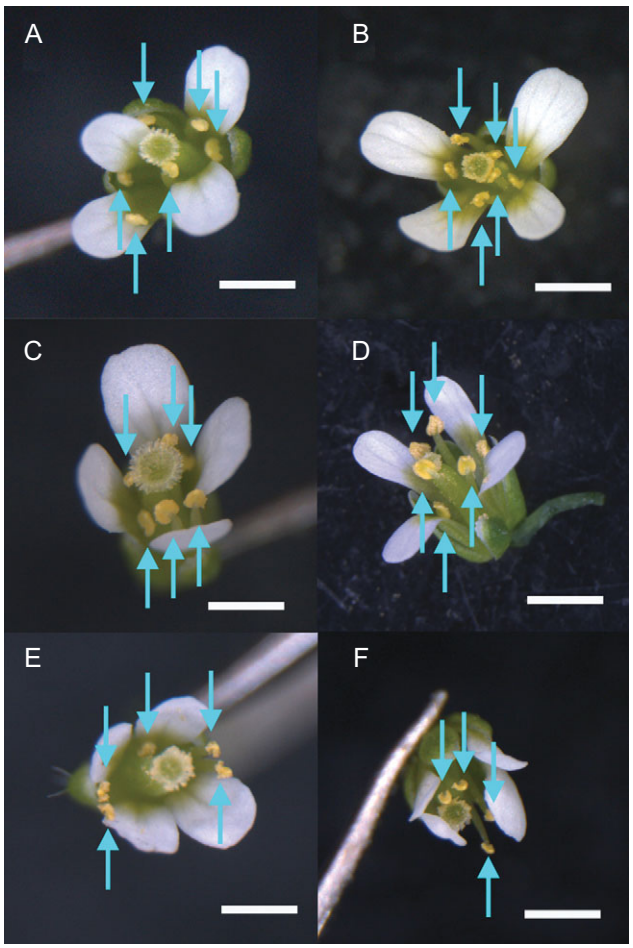

FIG. 6. Floral phenotype of the *AtEBP* knockout mutant. (A) Flower of WT, (B) vector control plant, (C, D) *AtEBP* over-expressing plants, and (E, F) *AtEBP* knockout plants. Each arrow indicates a stamen. Scale bars = 1 mm.

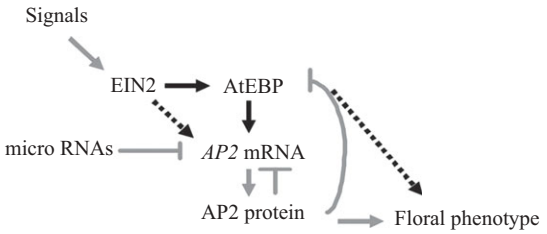

FIG. 7. Schematic diagram illustrating the relationship between AP2 and AtEBP. The translation of *AP2* mRNA is suppressed by micro RNAs (Chen, 2004) and AP2 protein down-regulates *AP2* and *AtEBP* expression (Okamuro *et al.*, 1997; this study), as indicated in grey. In the current study, it was demonstrated that *AP2* expression was regulated through AtEBP and EIN2, and that AtEBP may contribute to floral development, as indicated by in black.

signalling pathway under EIN2. It is known that the sensitivity of *ein3* mutants to ethylene is weaker than *ein2* mutants (Wang *et al.*, 2002). Previous studies reported that both EIN3-dependent and independent pathways exist downstream of EIN2 (Binder *et al.*, 2004; Seifert *et al.*, 2004). Furthermore, *AtEBP* expression is independently regulated under EIN3 in ethylene signalling (Ogawa *et al.*, 2005; this study). In this study, *AP2* was not induced by ethylene despite increasing expression of *AtEBP*. We suggest that these signal transductions compete with one another.

AtEBP knockout plants exhibited a weak floral phenotype with a lower number of stamens. An evaluation was also made of *AtERF1* and *AP2* expression in *AtEBP* knockout plants having the same level of WT (data not shown). The *ctr1* mutants showed an earlier-maturing phenotype in the gyneocium compared with the flower, and *ein* mutants affect the maturation of the gyneocium (Kieber *et al.*, 1993). Interestingly, the *ant* mutants show a similar phenotype to the *AtEBP* knockout plants (Elliott *et al.*, 1996; Klucher *et al.*, 1996). *ANT* is a member of the AP2/ERF family containing the AP2/EREBP domains.

Over-expression of *AtEBP* caused up-regulation of *AP2* in leaves. Despite the accumulation of *AP2* mRNA in transgenic *Arabidopsis* plants over-expressing *AtEBP*, no abnormal flowers were observed. Chen (2004) reported that micro RNAs control transcriptional regulation of *AP2* expression. That is, most transgenic *Arabidopsis* plants over-expressing *AP2* had normal flowers and only a fraction exhibited the *agamous*-like phenotype. However, over-expression of *AP2* mutated at the target site of micro RNAs demonstrated a more severe floral phenotype. Accumulation of AP2 protein was detected only in transgenic plants over-expressing mutated *AP2*, not in normal *AP2*. Thus, we consider that accumulation of *AP2* mRNA in *Arabidopsis* over-expressing *AtEBP* is not sufficient to change flower development.

This study has shown the mutual relationships between *AP2* and *AtEBP*. AtEBP and functional EIN2 affected the transcriptional regulation of *AP2*. AtEBP contributed slightly to flower development, especially stamen development. Future reports in this series will focus on the homeotic role of AtEBP.

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LITERATURE CITED

Alonso JM, Hirayama T, Roman G, Nourizadeh S, Ecker JR. 1999. EIN2, a bifunctional transducer of ethylene and stress responses in Arabidopsis. *Science* **284**: 2148–2152.

Aukerman MJ, Sakai H. 2003. Regulation of flowering time and floral organ identity by a MicroRNA and its *APETALA2*-like target genes. *Plant Cell* **15**: 2730–2741.

Binder BM, Mortimore LA, Stepanova AN, Ecker JR, Bleecker AB. 2004. Short–term growth responses to ethylene in *Arabidopsis* seedlings are EIN3/EIL1 independent. *Plant Physiology* **136**: 2921–2927.

Bomblies K, Dagenais N, Weigel D. 1999. Redundant enhancers mediate transcriptional repression of *AGAMOUS* by APETALA2. *Developmental Biology* **216**: 260–264.

Büttner M, Singh KB. 1997. *Arabidopsis thaliana* ethylene-responsive element binding protein (AtEBP), an ethylene-inducible, GCC box DNA-binding protein interacts with an ocs element binding protein. *Proceedings of the National Academy of Sciences of the USA* **94**, 5961–5966.

Chakravarthy S, Tuori RP, D'Ascenzo MD, Fobert PR, Despres C, Martin GB. 2003. The tomato transcription factor Pti4 regulates defense-related gene expression via GCC box and non-GCC box cis elements. *Plant Cell* **15**: 3033–3050.

Chen X. 2004. A microRNA as a translational repressor of APETALA2 in *Arabidopsis* flower development. *Science* **303**: 2022–2025.

Drews GN, Bowman JL, Meyerowitz EM. 1991. Negative regulation of the *Arabidopsis* homeotic gene *AGAMOUS* by the APETALA2 product. *Cell* **65**: 991–1002.

Elliott RC, Betzner AS, Huttner E, Oakes MP, Tucker WQ, Gerentes D, Perez P, Smyth DR. 1996. *AINTEGUMENTA*, an *APETALA2*-like gene of *Arabidopsis* with pleiotropic roles in ovule development and floral organ growth. *Plant Cell* **8**, 155–168.

Fujimoto SY, Ohta M, Usui A, Shinshi H, Ohme-Takagi M. 2000. *Arabidopsis* ethylene-responsive element binding factors act as transcriptional activators or repressors of GCC box-mediated gene expression. *Plant Cell* **12**, 393–404.

Jofuku KD, den Boer BG, Van Montagu M, Okamuro JK. 1994. Control of *Arabidopsis* flower and seed development by the homeotic gene *APETALA2*. *Plant Cell* **6**: 1211–1225.

Jofuku KD, Omidyar PK, Gee Z, Okamuro JK. 2005. Control of seed mass and seed yield by the floral homeotic gene *APETALA2*. *Proceedings of the National Academy of Sciences of the USA* **102**: 3117–3122.

Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR. 1993. CTR1, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of the raf family of protein kinases. *Cell* **72**: 427–441.

Klucher KM, Chow H, Reiser L, Fischer RL. 1996. The *AINTEGUMENTA* gene of *Arabidopsis* required for ovule and female gametophyte development is related to the floral homeotic gene *APETALA2*. *Plant Cell* **8**: 137–153.

Ogawa T, Pan L, Kawai-Yamada M, Yu LH, Yamamura S, Koyama T *et al.* 2005. Functional analysis of *Arabidopsis* ethylene-responsive element binding protein conferring resistance to bax and abiotic stress-induced plant cell death. *Plant Physiology* **138**: 1436–1445.

Ohto MA, Fischer RL, Goldberg RB, Nakamura K, Harada JJ. 2005. Control of seed mass by APETALA2. *Proceedings of the National Academy of Sciences of the USA* **102**: 3123–3128.

Okamuro JK, Caster B, Villarroel R, Van Montagu M, Jofuku KD. 1997. The AP2 domain of *APETALA2* defines a large new family of DNA binding proteins in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the USA* **94**: 7076–7081.

Riechmann JL, Heard J, Martin G, Reuber L, Jiang C, Keddie J *et al*. 2000. *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. *Science* **290**, 2105–2110.

Solano R, Stepanova A, Chao Q, Ecker JR. 1998. Nuclear events in ethylene signalling: a transcriptional cascade mediated by *ETHYLENE-INSENSITIVE3* and *ETHYLENE-RESPONSE-FACTOR1*. *Genes and Development* **12**: 3703–3714.

Seifert GJ, Barber C, Wells B, Roberts K. 2004. Growth regulators and the control of nucleotide sugar flux. *Plant Cell* **16**: 723–730.

Wang KL, Li H, Ecker JR. 2002. Ethylene biosynthesis and signalling networks. *Plant Cell* **14**: S131–151 (Supplement).

Weigel D, Meyerowitz EM. 1994. The ABCs of floral homeotic genes. *Cell* **78**: 203–209.