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The Transcriptional Co-activator MBF1c Is a Key Regulator of Thermotolerance in *Arabidopsis thaliana*[*S]

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Nobuhiro Suzuki[‡], Sunil Bajad[§], Joel Shuman[§], Vladimir Shulaev[§], and Ron Mittler[‡¶1]

From the [‡]Department of Biochemistry and Molecular Biology, University of Nevada, MS200, Reno, Nevada 89557, [§]Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, Virginia 24061, and the [¶]Department of Plant Sciences, Hebrew University of Jerusalem, Givat Ram, Jerusalem 91904, Israel

The ability of an organism to acclimate to its environment is a key determinant in its global distribution and capacity to compete with other organisms. The heat stress response, a highly conserved environmental and developmental program in eukaryotic and prokaryotic organisms, is an important component of the acclimation response of plants. Previous studies have shown that heat shock transcription factors play an important role in thermotolerance in plants and other organisms, controlling the expression of different heat shock proteins and detoxifying enzymes. In contrast, although several other pathways, involving ethylene, salicylic acid (SA), and trehalose, were recently shown to play a central role in thermotolerance in plants, a key regulator of these responses was not identified. Here we report that the highly conserved transcriptional co-activator, MBF1c (multiprotein bridging factor 1c), is a key regulator of thermotolerance in *Arabidopsis thaliana*. MBF1c protein accumulates rapidly and is localized to nuclei during heat stress. MBF1c is required for thermotolerance and functions upstream to SA, trehalose, ethylene, and pathogenesis-related protein 1 during heat stress. In contrast, MBF1c is not required for the expression of transcripts encoding HSFA2 and different heat shock proteins. Interestingly, MBF1c interacts with TPS5 (trehalose phosphate synthase 5), which is also heat-inducible, and mutants deficient in TPS5 are thermosensitive. Our results provide evidence for the existence of a tightly coordinated heat stress-response network, involving trehalose-, SA-, and ethylene-signaling pathways, that is under the control of MBF1c.

Thermotolerance is an essential component of the acclimation response of different organisms (1–6). It is generally divided into acquired thermotolerance (*i.e.* the ability to acquire tolerance to otherwise lethal heat stress) and basal thermotolerance (*i.e.* the inherent ability to survive temperature above the optimal growth) (5). Previous studies have shown that heat shock transcription factors (HSFs)[2] play an important role in thermotolerance in plants and other organisms, regulating heat shock proteins (HSPs) as well as different acclimation and detoxification proteins (7–10). In contrast, although several other pathways, involving ethylene, salicylic acid (SA), and trehalose, have recently been proposed to play an important role in thermotolerance in plants (5, 11–15), a key regulator of these responses was not identified.

MBF1 (multiprotein bridging factor 1) is a highly conserved transcriptional co-activator involved in the regulation of diverse processes, such as endothelial cell differentiation, hormone-regulated lipid metabolism, central nervous system development, and histidine metabolism (16–19). MBF1 proteins from different organisms interact with transcription factors, such as c-Jun, GCN4, and ATF1, or with different hormone receptors and link them with the TATA-binding protein (16–20). The flowering plant *Arabidopsis thaliana* contains three different genes encoding MBF1, all of which can complement MBF1 deficiency in yeast (21). MBF1a (At2g42680) and MBF1b (At3g58680) are developmentally regulated (22). In contrast, the expression of MBF1c (At3g24500) is elevated in *Arabidopsis* in response to pathogen infection, salinity, drought, heat, hydrogen peroxide, and application of the plant hormones abscisic acid or SA (21–23).

Here we report that MBF1c is a key regulator of thermotolerance in *Arabidopsis*. MBF1c is required for basal thermotolerance and functions upstream to SA, trehalose, and ethylene during heat stress. In contrast, MBF1c is not required for acquired thermotolerance or the expression of HSFA2 and different HSPs. Interestingly, MBF1c associates with a heat-induced protein thought to be involved in trehalose biosynthesis (TPS5 (trehalose phosphate synthase 5); At4g17770) and is localized to the nuclei of cells during heat stress.

EXPERIMENTAL PROCEDURES

Plant Material, Growth Conditions, and Stress Assays—A. thaliana cv. Columbia plants were grown under controlled conditions: 21 °C, 100 μmol m^{-2} s^{-1} (23). Null mutants for MBF1c (SALK_083813) and TPS5 (SALK_144791 and SALK_007952) were obtained, backcrossed, and obtained in homozygous form as described in Refs. 24–26. When grown

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. 1–3 and Table 1.

[1] To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Biology, University of Nevada, MS200, Reno, NV 89557. Tel.: 775-784-1384; Fax: 775-784-6911; E-mail: ronm@unr.edu.

[2] The abbreviations used are: HSF, heat shock transcription factor; HSP, heat shock protein; GFP, green fluorescent protein; ACC, 1-aminocyclopropane-1-caboxylic acid; SA, salicylic acid; MBF1, multiprotein bridging factor 1; TPS, trehalose phosphate synthase; RT, reverse transcription.

under controlled growth conditions, *mbf1c*, *tps5-1*, and *tps5-2* did not display any visible phenotype (not shown). Plant transformation, null mutant rescue, and selection were performed as described in Refs. 24–27. GFP or GFP fused in-frame to the C-terminal of MBF1c was expressed in plants under the control of the 35 S CaMV promoter, using a pGreen-based vector, and visualized using a Nikon Eclipse E400 epifluorescence microscope (Nikon Corp., Tokyo, Japan) or an Olympus, IX 81 FV 1000 conofocal microscope (Olympus, Tokyo, Japan), with 4′,6-diamidino-2-phenylindole as control for nuclei (28). Leptomycin B (2 μM) was used to inhibit nuclear export (29). T3 homozygous lines obtained from three individual transformation events, a homozygous null mutant, and two T3 complemented homozygous null mutants were used for all heat stress studies of MBF1c, and two independent homozygous null mutants were used for all heat stress studies of TPS5 (5, 23, 26). Basal and acquired thermotolerance were measured as described in Refs. 5 and 23. The effects of trehalose (1 mM), 1-aminocyclopropane-1-caboxylic acid (ACC) (10 μM), or SA (0.1 mM) on mutant survival were studied as described in Refs. 11 and 30–32, using a 2-h treatment at 45 °C.

For the analysis of transcript, protein, and metabolite accumulation, 2-week-old plants were subjected to 38 °C, 100 μmol m^{-2} s^{-1}, and sampled at 0, 10, 20, 30, and 60 min. Control plants kept at 21 °C were sampled in parallel. All experiments were performed in triplicates and repeated at least three times. Statistical analysis was performed as described in Ref. 23.

Molecular and Biochemical Analysis—RNA and protein were isolated and analyzed according to Refs. 24–26. cDNA probes corresponding to the following genes were used for RNA gel blots: PR1, At2g14610; TPS5, At4g17770; TPS11, At2g18700; Zat12, At5g59820; Zat7, At3g46080; APX1, At1g07890; HSFA2, At2g26150; HSP70, At3g12580; HSP90, At5g52640; HSP101, At1g74310. RT-PCR was performed according to Ref. 33, using the following primers for TPS5: 5′-TGTCAAGCCAAAGAGCTTATGGAAC, 3′-ATCATCACCGACGCAGAGAATGAAA. Metabolic analysis was performed as described in Refs. 23, 32, and 34 (supplemental materials). Yeast two-hybrid analysis was performed at the University of Wisconsin Molecular Interaction Facility, as described in Ref. 35. Testing for protein-protein interaction by growth on selection media and β-galactosidase activity was performed as described in Ref. 35. Analysis of microarray data, available on the World Wide Web, was performed as previously described (36). The following annotation for TPS was used: TPS1, At1g78580; TPS2, At1g16980; TPS3, At1g17000; TPS4, At4g27550; TPS5, At4g17770; TPS6, At1g68020; TPS7, At1g06410; TPS8, At1g70290; TPS9, At1g23870; TPS10, At1g60140; TPS11, At2g18700.

Protein Purification and Antibody Production—The coding region of MBF1c was expressed in *Escherichia coli* BL21 Gold cells using the pCAL-n vector (Stratagene, La Jolla, CA). MBF1c expression was induced by 1 mM isopropyl 1-thio-β-D-galactopyranoside in 1 liter of liquid LB medium for 3 h at 37 °C. Cells were harvested, and MBF1c was purified from inclusion bodies (37), resuspended in 1 M Tris-HCl (pH 8.0), and separated by 12% SDS-PAGE. MBF1c protein was cut from gels and used for the production of rabbit polyclonal antibodies (AnaSpec Inc., San Jose, CA).

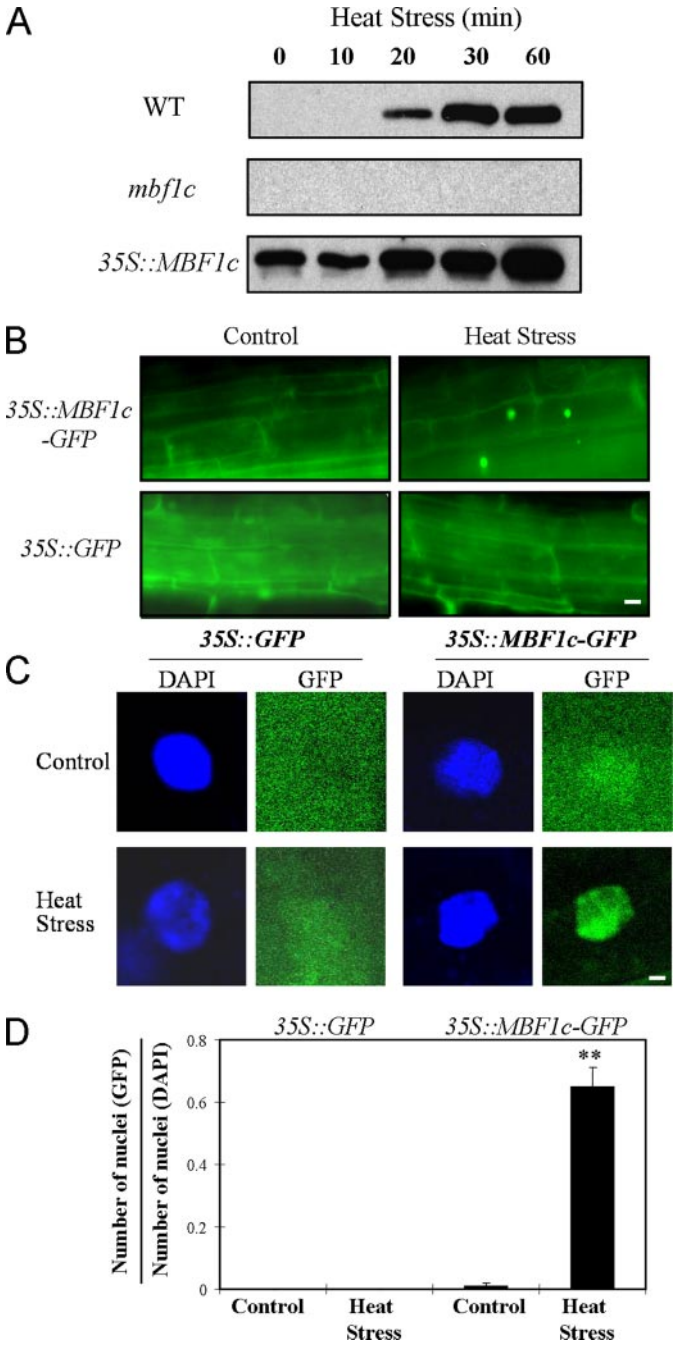

FIGURE 1. **Accumulation of MBF1c protein and localization of MBF1c-GFP fusion protein to nuclei during heat stress in *Arabidopsis*.** *A*, protein blots of MBF1c accumulation during heat stress (38 °C) in wild type (*WT*), *mbf1c*, and *35S::MBF1c* plants. *B*, images of root cells from *35S::GFP* or *35S::MBF1c-GFP* plants grown under controlled growth conditions (*Control*) or subjected to a 1-h heat stress (*Heat Stress*; 38 °C). *C*, enlarged images of root cells from *35S::GFP* or *35S::MBF1c-GFP* plants grown under controlled growth conditions or subjected to a 1-h heat stress. *D*, quantification of the nuclear localization data in root cells. At least 150 nuclei were counted for each group. Nuclear localization was confirmed with 4′,6-diamidino-2-phenylindole (*DAPI*). Similar results were obtained with leaf cells (not shown). **, *t* test significant at $p < 0.01$.

RESULTS

Characterization of MBF1c Protein in Arabidopsis—MBF1c protein rapidly accumulated in *Arabidopsis* leaves during heat stress (Fig. 1*A*). In contrast, MBF1c protein was not detected in wild type plants grown under controlled conditions or in a null

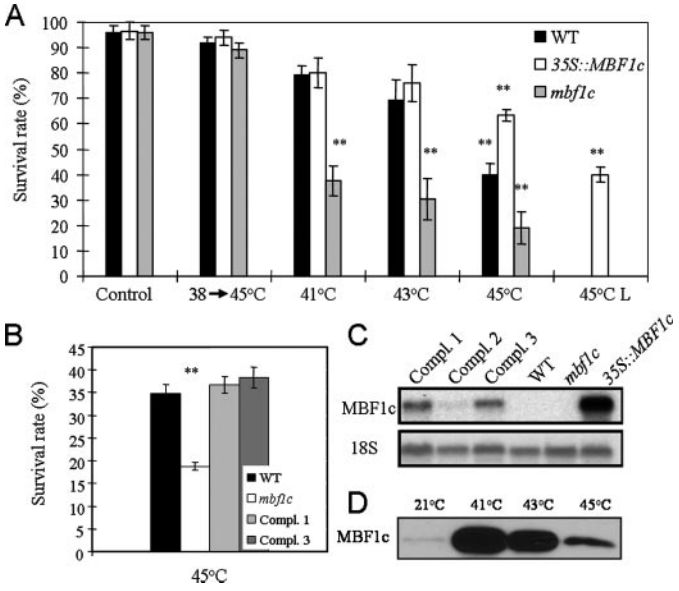

FIGURE 2. **Thermotolerance of gain- and loss-of-function MBF1c plants.** *A*, survival of 5-day-old wild type (*WT*), *mbf1c*, and *35S::MBF1c* seedlings directly subjected to heat stress (41, 43, or 45 °C) for 2 h to measure basal thermotolerance or treated at 38 °C for 1.5 h, allowed to recover for 1 h at 21 °C, and subjected to 45 °C for 2 h (38 → 45 °C) to measure acquired thermotolerance. Seedlings were also kept at 45 °C until all wild type plants died (45 °C L). *B*, rescue of *mbf1c* with low level constitutive expression of MBF1c (*Compl. 1* and *Compl. 3* represent two individual *mbf1c* transgenic plants transformed with the *35S::MBF1c* construct). *C*, RNA blots showing MBF1c expression in *mbf1c* transgenic plants transformed with MBF1c (*Compl. 1* and *3* were used in *B*; *Compl. 2* was unable to rescue the mutant; not shown). The expression of MBF1c in the different lines is compared with that of MBF1c in the transgenic plants used in Ref. 23 and identified in this paper as *35S::MBF1c*. *D*, protein blot showing expression of MBF1c in wild type seedlings subjected to heat stress (41, 43, or 45 °C) for 2 h and used to measure basal thermotolerance. **, *t* test significant at $p < 0.01$.

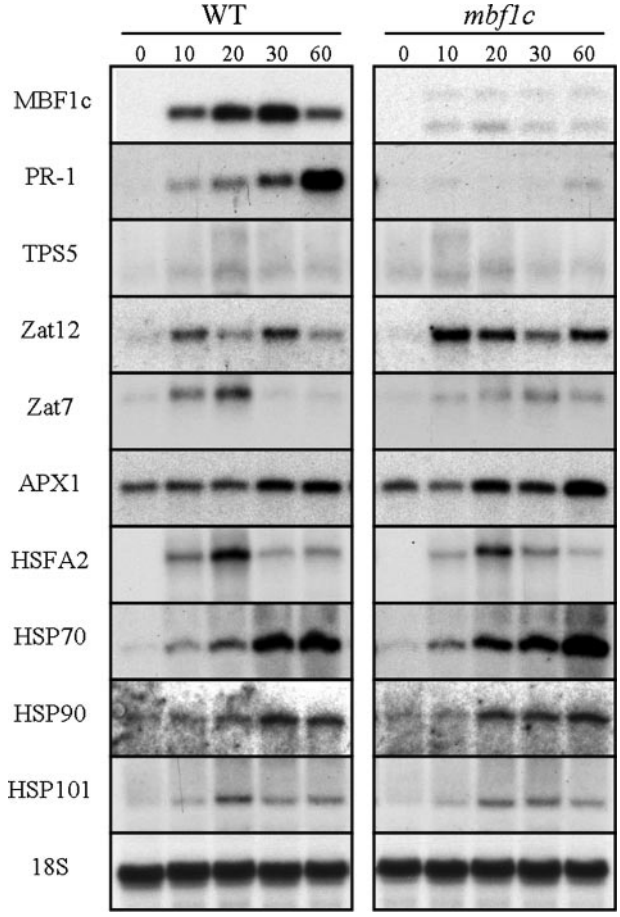

FIGURE 3. **Expression of transcripts encoding different regulatory and acclimation proteins in loss-of-function MBF1c plants during heat stress.** RNA gel blots were performed on samples obtained from wild type (*WT*) and *mbf1c* plants at different times (min) following heat stress (38 °C). Ribosomal 18 S RNA was used to control for RNA loading.

mutant of MBF1c (*mbf1c*; SALK_083813). MBF1c protein was detected in transgenic plants constitutively expressing MBF1c (*35S::MBF1c*), grown under controlled conditions, or subjected to heat stress (Fig. 1*A*). The size of MBF1c protein detected by protein blots in *Arabidopsis* plants precisely corresponded to the predicted molecular weight of the MBF1c open reading frame (16.4 kDa; not shown).

To examine subcellular localization of MBF1c, MBF1c-GFP fusion protein (C-terminal fusion) and control GFP protein were expressed in transgenic plants under the control of the 35 S CaMV promoter (*35S::MBF1c-GFP* and *35S::GFP*, respectively). When transgenic plants with constitutive expression of MBF1c-GFP were grown under controlled conditions, MBF1c-GFP fusion protein was mainly localized to the cytosol (Fig. 1, *B–D*). In contrast, when plants were subjected to heat stress (1 h, 38 °C), MBF1c-GFP protein was mainly detected in nuclei, whereas the localization of the GFP control protein was not altered (Fig. 1, *B–D*). This response, observed with three independent transgenic lines expressing MBF1c-GFP at different levels (not shown), was not effected by leptomycin B (2 μM), an inhibitor of nuclear export, suggesting that nuclear localization of MBF1c during heat stress is a result of enhanced nuclear import (not shown).

Thermotolerance of Knock-out Plants Deficient in MBF1c Expression—A null mutant of MBF1c (*mbf1c*; SALK_083813) was isolated and subjected to heat stress. *mbf1c* did not accumulate MBF1c protein in response to heat stress (Fig. 1*A*). Wild type, *mbf1c*, and *35S::MBF1c* seedlings were directly subjected to heat stress (41, 43, or 45 °C) for 2 h to measure basal thermotolerance or treated at 38 °C for 1.5 h, allowed to recover for 1 h at 21 °C, and subjected to 45 °C for 2 h (38 → 45 °C), to measure acquired thermotolerance. *mbf1c* plants were not deficient in acquired thermotolerance (38 → 45 °C) but were deficient in basal thermotolerance at different temperatures (41, 43, or 45 °C; Fig. 2*A*). In contrast to *mbf1c*, or wild type plants, *35S::MBF1c* plants were more tolerant to basal thermotolerance at 45 °C (Fig. 2*A*). This was also evident when the 45 °C treatment was maintained for longer than 2 h until all wild type plants died (45 °C L). The basal thermotolerance phenotype of the MBF1c null mutant (*mbf1c*) could be rescued by a low level of constitutive MBF1c expression (Fig. 2, *B* and *C*). As shown in Fig. 2*D*, MBF1c protein accumulated in wild type seedlings subjected to heat stress (41, 43, or 45 °C) for 2 h and used to measure basal thermotolerance (Fig. 1*A*).

Transcript Expression in MBF1c Null Mutants during Heat Stress—Transcript expression of MBF1c was enhanced as early as 10 min following heat stress application (Fig. 3). In contrast, MBF1c transcript was not detected in *mbf1c* plants. Compared with wild type, transcript expression of HSFA2, several different HSPs, and cytosolic APX1 (ascorbate peroxidase 1) was not

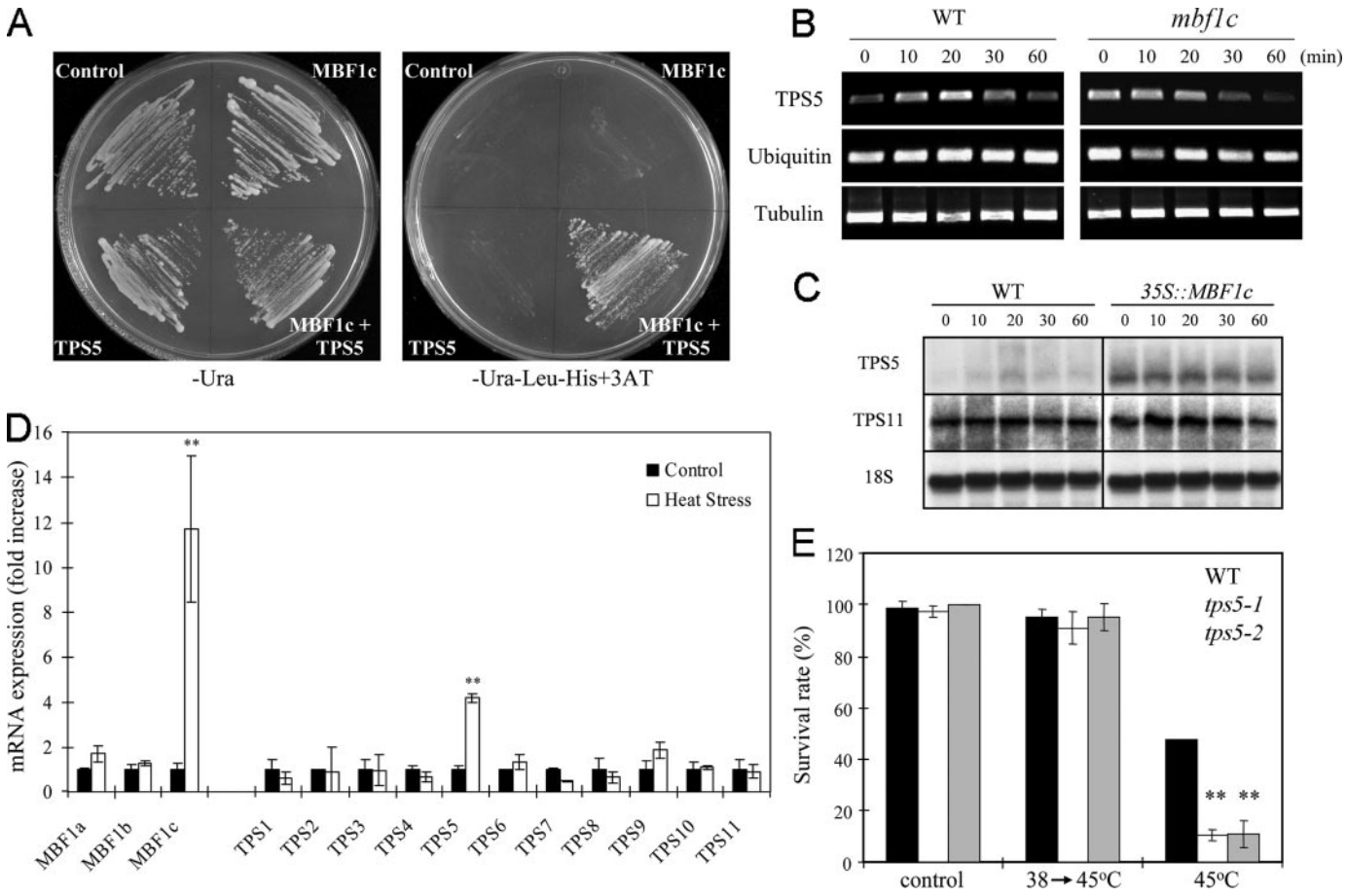

FIGURE 4. **Interaction of MBF1c with TPS5.** *A*, survival of yeast co-expressing MBF1c and TPS5 on selection media, indicating protein-protein interaction. Full-length cDNAs of MBF1c and TPS5 were cloned into pBUTE bait and pGADT7-REC prey vectors, respectively, and transformed into a PJ69-4A yeast strain. Interaction was tested by growing yeast on −Ura, −Leu, −His media containing 1 mM 3-amino-1,2,4-triazole (*3-AT*). *B*, TPS5 mRNA expression in wild type (*WT*) and *mbf1c* plants during heat stress (38 °C) measured by RT-PCR. Ubiquitin and tubulin were used as internal controls. *C*, TPS5 mRNA expression in wild type and *35S::MBF1c* plants during heat stress (38 °C) measured by RNA gel blots. *D*, relative expression of all MBF1 and TPS transcripts in *Arabidopsis* during heat stress. Expression data were obtained from the World Wide Web (Ref. 38). *E*, thermotolerance of 5-day-old wild type *tps5-1* (SALK_144791) and *tps5-2* (SALK_007952) seedlings. **, *t* test significant at $p < 0.01$.

altered in *mbf1c* plants during heat stress (Fig. 3). In contrast, PR-1 (pathogenesis-related protein 1) transcript expression was not detected in *mbf1c* plants during heat stress, and the expression of transcripts encoding the reactive oxygen species response zinc finger protein Zat12 (24–26) was enhanced in *mbf1c* plants compared with wild type plants during heat stress. In contrast to Zat12 and compared with wild type plants, the expression of Zat7, which functions as a repressor (35), decreased in *mbf1c* plants during heat stress (Fig. 3). The rapid expression of MBF1c during heat was not suppressed in mutants deficient in SA (*npr1*) or chloroplast-to-nuclei retrograde signaling (*gun1*, *abi4*, and *tylapx*; supplemental Fig. 1).

Interaction of MBF1c with TPS5—A yeast two-hybrid screen was performed for the full-length cDNA clone of MBF1c. As prey, we used a library constructed from *Arabidopsis* seedlings subjected to different abiotic stresses (35). Approximately 18 million clones were screened via mating. Of these, 52 tested positive for interaction. Isolates were then validated by retransformation and one-on-one mating, followed by selection and growth and β-galactosidase assays (35) (supplemental Table 1). At4g17770 (TPS5); also contains an intact C-terminal trehalose phosphatase domain) and two proteins (At3g12960 and At1g43170) that are most likely yeast two-hybrid artifacts (35) were identified.

MBF1c could interact with TPS5 *in vivo* in yeast (Fig. 4*A*) as well as *in vitro* (supplemental Fig. 2). Transcript expression of TPS5 was too low to be detected by RNA blots in wild type and *mbf1c* plants during heat stress (Fig. 3). RT-PCR analysis was therefore used and showed a transient increase in TPS5 expression during heat stress in wild type plants (Fig. 4*B*). In contrast, TPS5 expression was high in *mbf1c* plants in the absence of stress but declined during heat stress (Fig. 4*B*). Interestingly, the steady-state transcript level of TPS5 was elevated in *35S::MBF1c* plants in the presence or absence of heat stress (Fig. 4*C*). *In silico* analysis of all TPS transcripts in *Arabidopsis* (36, 38) revealed that TPS5 is the only TPS transcript elevated during heat stress in *Arabidopsis* (Fig. 4*D*). Moreover, two independent TPS5 null mutants (SALK_144791, *tps5-1*; SALK_007952, *tps5-2*) were deficient in basal (45 °C) but not acquired (38 → 45 °C) thermotolerance (Fig. 4*E*).

Involvement of MBF1c in Trehalose Metabolism during Heat Stress—Trehalose accumulated in wild type plants during heat stress and in *35S::MBF1c* plants grown under controlled conditions (Fig. 5, *A* and *C*). In contrast, trehalose did not accumulate

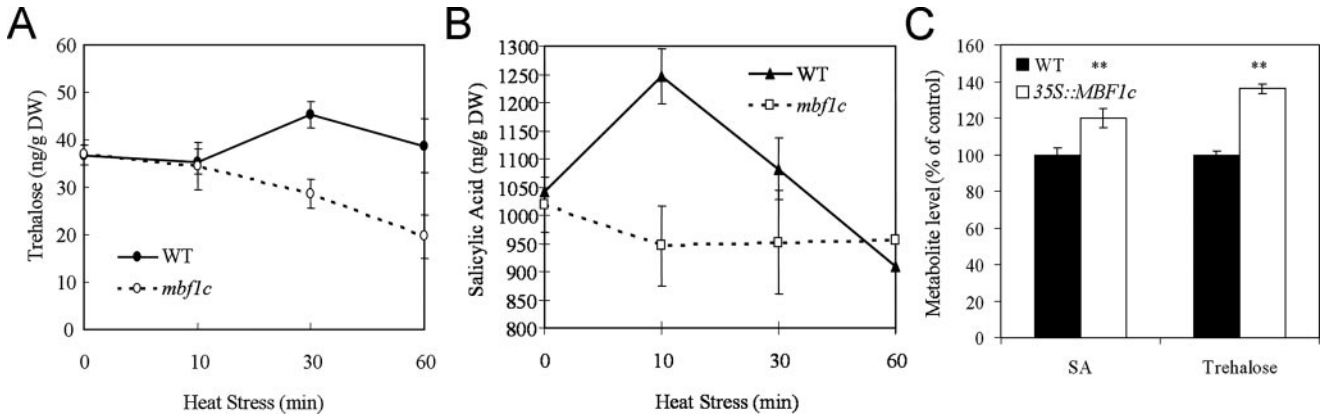

FIGURE 5. **Trehalose and SA accumulation in *mbf1c* and *35S::MBF1c* plants.** *A*, measurements of trehalose in wild type (WT) and *mbf1c* plants at different times during heat stress (38 °C). *B*, measurements of SA in wild type and *mbf1c* plants at different times during heat stress (38 °C). *C*, measurements of trehalose and SA in wild type and *35S::MBF1c* plants grown under controlled growth conditions. **, *t* test significant at $p < 0.01$.

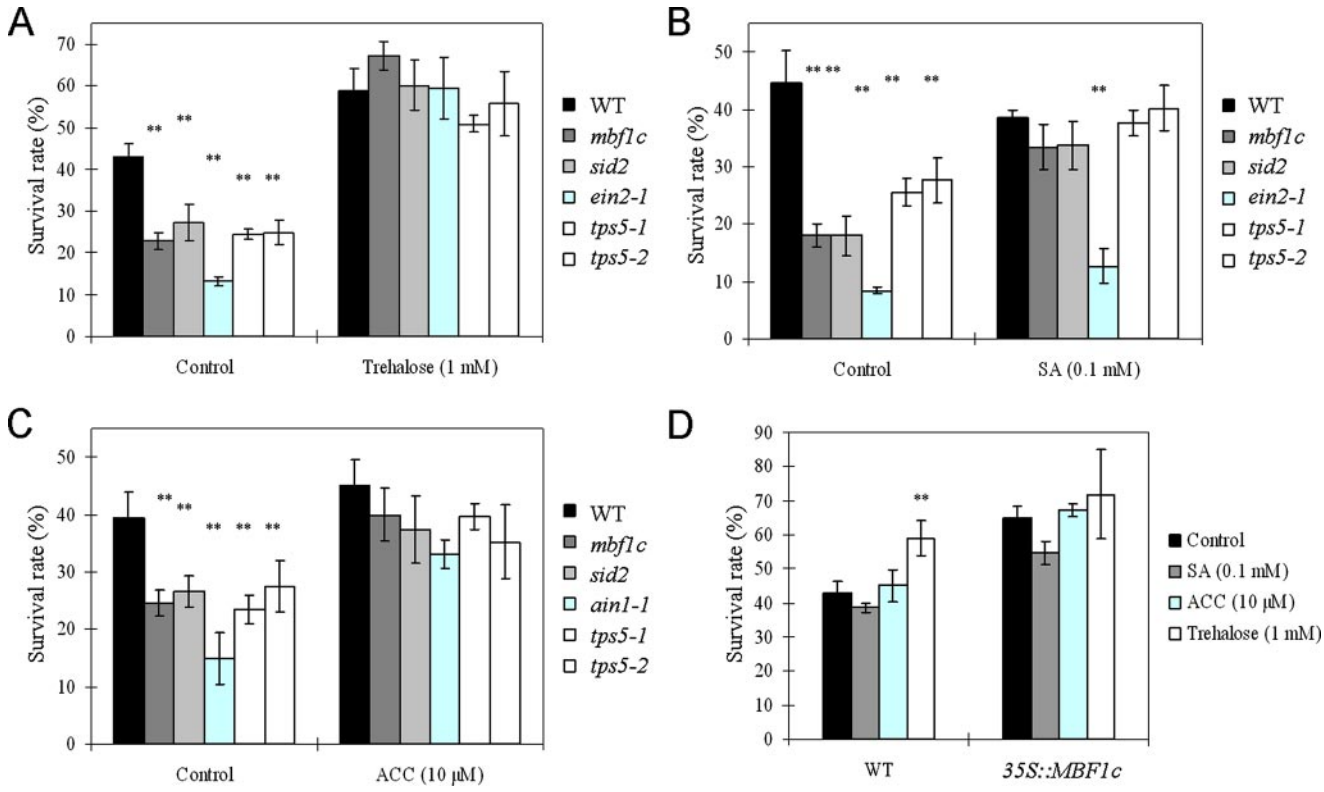

FIGURE 6. **Rescue of *mbf1c* during heat stress by trehalose, SA, and ACC.** *A*, rescue of *mbf1c*, *sid2*, *ein2-1*, and *tps5-1* and *tps5-2* during heat stress by trehalose (1 mM). *B*, rescue of *mbf1c*, *sid2*, *ein2-1*, and *tps5-1* and *tps5-2* during heat stress by SA (0.1 mM). *C*, rescue of *mbf1c*, *sid2*, *ain1-1*, and *tps5-1* and *tps5-2* during heat stress by ACC (10 μM). *D*, rescue of *35S::MBF1c* plants during heat stress by trehalose (1 mM), SA (0.1 mM), or ACC (10 μM). Seedlings were directly subjected to heat stress (45 °C, 2 h) in the presence or absence of trehalose, SA, or ACC and scored for survival. **, *t* test significant at $p < 0.01$.

during heat stress in *mbf1c* plants (Fig. 5*A*), suggesting that MBF1c functions upstream to trehalose accumulation during heat stress. We therefore examined whether trehalose will rescue *mbf1c* plants from heat stress. Trehalose (1 mM) was able to rescue *mbf1c* plants as well as *tps5-1* and *tps5-2* and mutants deficient in ethylene (*ein2-1*) and SA (*sid2*) (39) signaling (Fig. 6*A*). *mbf1c* and *ein2* plants were both deficient in basal thermotolerance, supporting our previous studies on MBF1c and ethylene signaling (23). In contrast to the results obtained with *mbf1c* or *ein2* plants, trehalose did not alter the heat tolerance of transgenic plants that constitutively express MBF1c (*35S::MBF1c*; Fig. 6*D*), suggesting that the trehalose-dependent enhancement of thermotolerance observed in *mbf1c*, *tps5-1*, *tps5-2*, and *ein2-1* and *sid2* plants is already activated in *35S::MBF1c* plants. This possibility is also supported by the findings that trehalose improved the basal thermotolerance of wild type plants and brought it to the level found in *35S::MBF1c* plants (Fig. 6*D*) and that *35S::MBF1c* plants accumulated trehalose (Fig. 5*C*).

MBF1c Functions Upstream to SA during Heat Stress—PR-1 expression was similar in *mbf1c* and wild type plants 3 h following SA application (supplemental Fig. 3), suggesting that MBF1c is not directly required for PR-1 expressing. SA transiently accumulated in wild type plants during heat stress and

accumulated in *35S::MBF1c* plants grown under controlled conditions (Fig. 5, *B* and *C*). In contrast, SA did not accumulate in *mbf1c* plants during heat stress (Fig. 5*B*), suggesting that MBF1c is required for SA accumulation during heat stress in *Arabidopsis*. SA (0.1 mM) was able to rescue *mbf1c* plants as well as a mutant deficient in SA accumulation (*sid2*) and *tps5-1* and *tps5-2* (Fig. 6*B*). *sid2* and *mbf1c* plants had a similar level of deficiency in basal thermotolerance, supporting our hypothesis that MBF1c functions upstream to SA during heat stress. In contrast to the effect of SA on the basal thermotolerance of *sid2*, SA failed to rescue the *ein2* mutant from heat stress (Fig. 6*B*). This result suggested that ethylene functions downstream to or independent of SA in the basal thermotolerance pathway of *Arabidopsis*. SA did not enhance the tolerance of transgenic plants that constitutively express MBF1c (*35S::MBF1c*) to heat stress (Fig. 6*D*), and these plants were found to accumulate SA when grown under controlled conditions (Fig. 5*C*).

SA Functions Upstream to Ethylene during Heat Stress—ACC (10 μM), an ethylene precursor, rescued *mbf1c*, *sid2*, *tps5-1*, *tps5-2*, and a mutant deficient in ethylene accumulation (*ain1-1*) (40) from heat stress (Fig. 6*C*). In contrast, ACC had no effect on *35S::MBF1c* plants (Fig. 6*D*). PR-1 expression could not be detected in *sid2* plants during heat stress (Fig. 7*A*). In contrast, PR-1 expression was detected in *ein2* plants during heat stress. Our results therefore support a model in which MBF1c functions upstream to SA accumulation that functions upstream to ethylene and PR-1 during heat stress. The expression of PR-1 during heat stress does not appear, however, to be dependent on ethylene. PR-1 expression is suppressed in *mbf1c* and *sid2* plants during heat stress (Figs. 3 and 7*A*). In contrast, PR-1 expression was not suppressed in *tps5* plants (Fig. 7*B*). This finding suggested that PR-1 expression is independent of TPS5 and that SA, ethylene, and PR-1 function separately from TPS5 during heat stress (Fig. 7*C*). In contrast to PR-1 expression, expression of HSFA2 was elevated in *tps5* mutants during heat stress (Fig. 7*B*), suggesting that *tps5* plants are attempting to compensate for their enhanced thermosensitivity (Fig. 4*E*) by overactivating the heat shock response pathway.

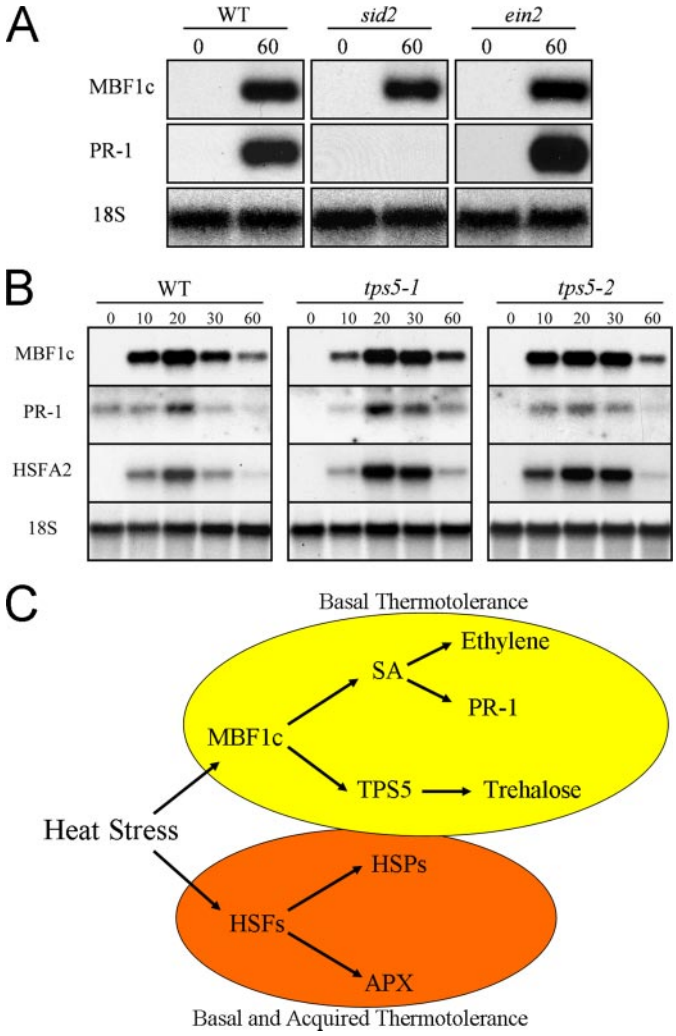

FIGURE 7. **A simplified model for the role of MBF1c during heat stress in *Arabidopsis*.** *A*, PR-1 transcript expression in *sid2* and *ein2-1* during heat stress (38 °C, 1 h). *B*, MBF1c, HSFA2, and PR-1 transcript expression in *tps5-1* and *tps5-2* during heat stress (38 °C). *C*, a model for MBF1c function. MBF1c is proposed to function upstream to SA, ethylene, and trehalose during heat stress. In contrast, MBF1c is proposed not to be required for the expression of HSFA2, APX1, and HSPs during heat stress.

DISCUSSION

A simplified model depicting the proposed role of MBF1c in regulating thermotolerance in *Arabidopsis* is shown in Fig. 7*C*. Our data support a model in which MBF1c functions upstream to trehalose, SA, and ethylene. Although our results support a model in which SA and ethylene function independently of trehalose (Fig. 7), it is not entirely clear at this point whether this model is completely accurate or whether a linear model, such as MBF1c → SA → ethylene → trehalose, is more suitable.

Similar to HSFs, MBF1 proteins are highly conserved in different organisms, including human, fly, yeast, and plants (7, 16–19). Our findings, therefore, raise an interesting possibility that MBF1 proteins from other organisms are also involved in regulating responses to temperature stress. The intimate relationship identified in our study between MBF1c and trehalose metabolism during heat stress (Figs. 4–7) could also be studied in other organisms, because trehalose is thought to play a key signaling and defense role during different environmental stress conditions (12, 15, 41–44). At least in plants, it is generally believed that trehalose functions as a signaling molecule (12, 15, 42, 45, 46). Previous studies have focused on TPSs as key regulators of trehalose signaling (12, 15). Although TPS belongs to a large gene family, only transcripts encoding TPS5 were found to specifically accumulate during heat stress, and mutants of TPS5 were deficient in basal thermotolerance (Fig. 4). These findings point to a high degree of specificity in the function of different TPS genes in *Arabidopsis* and suggest that specific TPS genes could have specialized roles in trehalose signaling during different growth conditions or environmental stresses.

Several lines of evidence support the hypothesis that MBF1c and TPS5 interact. The expression of TPS5 and MBF1c is co-elevated during heat stress (Fig. 4*D*), TPS5 and MBF1c were found to interact in yeast and *in vitro* (Fig. 4*A* and supplemental Fig. 2), null mutants for TPS5 and MBF1c are both sensitive to basal, but not acquired, thermotolerance (Figs. 2 and 4*E*), MBF1c null mutant has abnormal expression of TPS5 and does

not accumulate trehalose during heat stress (Figs. 4*B* and 5*A*), *35S*::*MBF1c* plants accumulate transcripts encoding TPS5 and trehalose (Figs. 4*C* and 5*C*), and trehalose can rescue *mbf1c* plants from heat stress (Fig. 6*A*). Nonetheless, additional studies are required to examine the possibility that MBF1c regulates trehalose metabolism via its interaction with TPS5.

MBF1c is thought to be a transcriptional co-activator. It was shown to complement MBF1 deficiency in yeast (21), suggesting that at least in yeast it could mediate transcription of GCN4-controlled genes. The localization of MBF1c protein to nuclei during stress (Fig. 1*B*) and the presence of a conserved helix-turn-helix DNA-binding domain in MBF1c (22), strengthen the possibility that MBF1c functions in plants as a transcriptional regulator. We previously performed transcriptome analysis of *35S*::*MBF1c* plants, grown under controlled growth conditions, and found that a large proportion of the transcripts elevated in *35S*::*MBF1c* plants were associated with ethylene, SA, and trehalose (23). These findings support our current analysis of *mbf1c* plants and suggest that MBF1c could function as a transcriptional regulator that enhances the expression of selected transcripts in plants in the absence of stress. It is therefore possible that even the low levels of MBF1c protein found in the nuclei of *35S*::*MBF1c-GFP* plants, grown under controlled growth conditions (Figs. 1, *B–D*), could activate ethylene, SA, and trehalose responses.

Our analysis of gain- and loss-of-function MBF1c plants provides strong evidence for a link between MBF1c and trehalose metabolism, suggests a role for MBF1c as a central regulator of thermotolerance in *Arabidopsis*, and demonstrates that MBF1c functions upstream to SA, PR-1, ethylene, and trehalose during heat stress. Because the expression of MBF1c is enhanced in response to many different conditions (21–23), it is possible that the role of MBF1c in the response of plants to different stresses is much wider than that depicted in our current work. In support of this possibility, *35S*::*MBF1c* plants were found to be more tolerant to heat and/or osmotic stresses as well as to bacterial infection (23).

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