Heat Shock Factor HsfB1 Primes Gene Transcription and Systemic Acquired Resistance in Arabidopsis[1][W]

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Over the past decade, plant heat shock factors (Hsfs) have been associated mainly with responses to abiotic stress (von Koskull-Döring et al., 2007), yet, until this report, they have not been linked to primed defense gene activation or pathogen-induced systemic acquired resistance (SAR). Hsfs are a diverse group of proteins that regulate genes encoding heat shock proteins (von Koskull-Döring et al., 2007). They do so by binding to a regulatory DNA motif called the heat stress element (Pelham, 1982). Arabidopsis (*Arabidopsis thaliana*) has more than 20 Hsfs that have been divided into three classes (A, B, and C) based on the amino acid sequence of their flexible linkers and oligomerization domains (HR-A/B regions; Nover et al., 2001). Although members of class A Hsfs seem to be bona fide transcription coactivators, class B Hsfs do not contain a transcription-promoting, C-terminal activation domain (Nover et al., 2001). However, they still can serve as transcription coactivators (Bharti et al., 2004).

A recent report with Arabidopsis revealed that HsfB1 (also referred to as HSF4 or TBF1) represses the general heat shock response in the absence of excessive heat but supports the development of acquired thermotolerance in times of heat stress (Ikeda et al., 2011). HsfB1 was also reported to be a negative regulator of the jasmonic acid/ethylene-responsive defensin genes *PDF1.2a* and *PDF1.2b* and to suppress the innate immune response of Arabidopsis to the necrotrophic fungus *Alternaria brassicicola* (Kumar et al., 2009). AtGenExpress microarray data suggested also a positive regulatory role for HsfB1 in the plant response to pathogens and microbe-associated molecular patterns

(von Koskull-Döring et al., 2007), but this has not been investigated further. Moreover, except for one very recent report (Pajerowska-Mukhtar et al., 2012), HsfB1 and other Hsfs have not been associated with SAR. This type of plant immune response is induced upon localized infection with a necrotizing pathogen and develops systemically throughout the plant (Hammerschmidt, 2009). SAR is associated with the systemic accumulation of the plant hormone salicylic acid, which primes the plant for a more robust and rapid activation of defense genes upon further pathogen attack (Conrath et al., 2006). Here, we show that HsfB1 positively regulates the primed expression of defense-related genes and that it is required for bona fide SAR to bacterial pathogens in Arabidopsis.

To investigate whether HsfB1 could play a role in the development of defense priming and SAR, we first assessed whether benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) could induce the expression of the *HsfB1* gene in Arabidopsis. BTH is a synthetic mimic of salicylic acid and a potent inducer of priming and SAR in Arabidopsis (Lawton et al., 1996; Beckers et al., 2009).

As shown in Figure 1, treatment of Arabidopsis plants with a formulation of BTH caused diurnal expression of the *HsfB1* gene, with *HsfB1* being activated in the light and dropping to basal expression levels in the dark. When plants were treated with a blank formulation devoid of BTH, little *HsfB1* expression was observed in both the light and dark periods (Fig. 1). Together, these results supported our assumption that HsfB1 plays a role in BTH-induced defense priming of Arabidopsis and suggested that HsfB1's role in BTH-induced defense priming depends on light. The latter conclusion is consistent with earlier findings demonstrating that SAR (Zeier et al., 2004; Griebel and Zeier, 2008), as well as the contribution of methyl salicylate to SAR (Liu et al., 2011), are light dependent.

To elucidate whether HsfB1 indeed is required for the primed expression of defense-related genes, and to provide genetic evidence for this, we included the Arabidopsis *hsfb1-1* (Ikeda et al., 2011) and *hsfb1-3* (Supplemental Fig. S1) mutants in our analyses. We investigated whether *hsfB1-1* and *hsfB1-3* would or would not display primed expression of the defense-related genes encoding Phe ammonia lyase 1 (PAL1) and WRKY29. The PAL enzyme catalyzes the committed step in the phenylpropanoid pathway, with
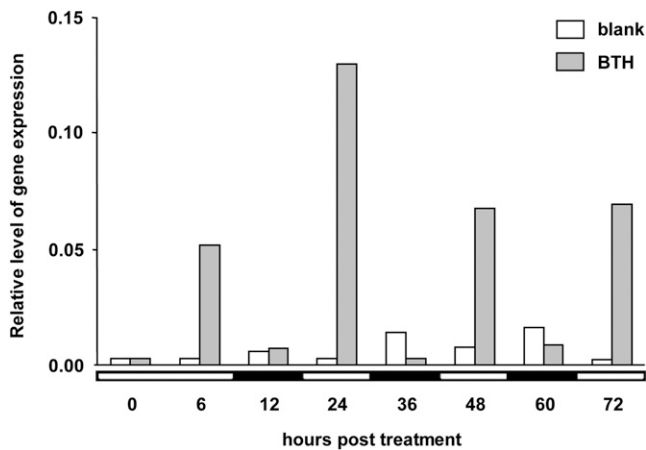
Figure 1. Diurnal activation of the *HsfB1* gene by BTH. Five-week-old Arabidopsis (accession Columbia-0) plants were grown on soil at 8 h of light/16 h of dark, 20°C, and 60% to 70% relative humidity. Plants were sprayed at 10 AM (that is 2 h after start of the light period) with a formulation of BTH (100 μM) or with a blank formulation devoid of BTH (blank). At various times after treatment, RNA was extracted from sprayed leaves and assayed for the accumulation of *HsfB1* transcripts by quantitative reverse transcription (qRT)-PCR using gene-specific primers (Supplemental Table S1) as described by Beckers et al. (2009). Relative amounts were normalized to those of *ACTIN2*. Bars represent means of two replicate measurements (*n* = 2). White and black bars below the *x* axis indicate whether the harvest of leaves was done in the light or the dark. The experiment was repeated two times with similar results.

important roles in the overall plant defense response (Hahlbrock and Scheel, 1989), whereas *WRKY29* encodes a transcription coactivator that is crucial for the regulation of plant defense genes (Eulgem and Somssich, 2007). These two loci were previously identified as reliable marker genes for the Arabidopsis stress response, and it was shown that wound stress-induced *PAL1* and *WRKY29* expression was more robust in primed and subsequently wounded plants than in wounded plants without previous priming (Kohler et al., 2002; Beckers et al., 2009; Jaskiewicz et al., 2011).

Wild-type plants (Columbia-0) and the *hsfb1-1* and *hsfb1-3* mutants were treated with BTH or the blank formulation for 3 d. Then, leaves were either left untreated or infiltrated with water. Water infiltration into leaves elicits a cell-collapse response and activates the expression of wound-responsive genes (Young et al., 1996; Kohler et al., 2002). Three hours after leaf infiltration, RNA was extracted from leaves and assayed for the accumulation of transcripts encoding PAL1 (Fig. 2A) or WRKY29 (Fig. 2B).

In wild-type Arabidopsis, pretreatment with BTH primed the infiltration-activated expression of both *PAL1* and *WRKY29* almost 3-fold when compared with the response in plants treated with the blank formulation without BTH (Fig. 2). By contrast, pretreatment with BTH did not significantly prime the infiltration-induced *PAL1* and *WRKY29* expression in

leaves of the *hsfb1-1* and *hsfb1-3* mutants (Fig. 2). However, the two mutants responded to the infiltration stimulus itself to a similar extent as wild-type plants (Fig. 2). Thus, the perception of and responsiveness to water infiltration per se seem not to be affected in *hsfb1-1* and *hsfb1-3*, but HsfB1 is required for priming.

In addition to abiotic stress tolerance, primed defense gene activation has been associated with SAR to pathogen attack (Conrath et al., 2006; Jung et al., 2009; Conrath, 2011). Furthermore, HsfB1 was recently found to be important for salicylic acid-induced disease resistance in Arabidopsis (Pajerowska-Mukhtar et al., 2012). Therefore, we investigated whether HsfB1, in addition to conferring acquired thermotolerance (Ikeda et al., 2011), chemically activated resistance
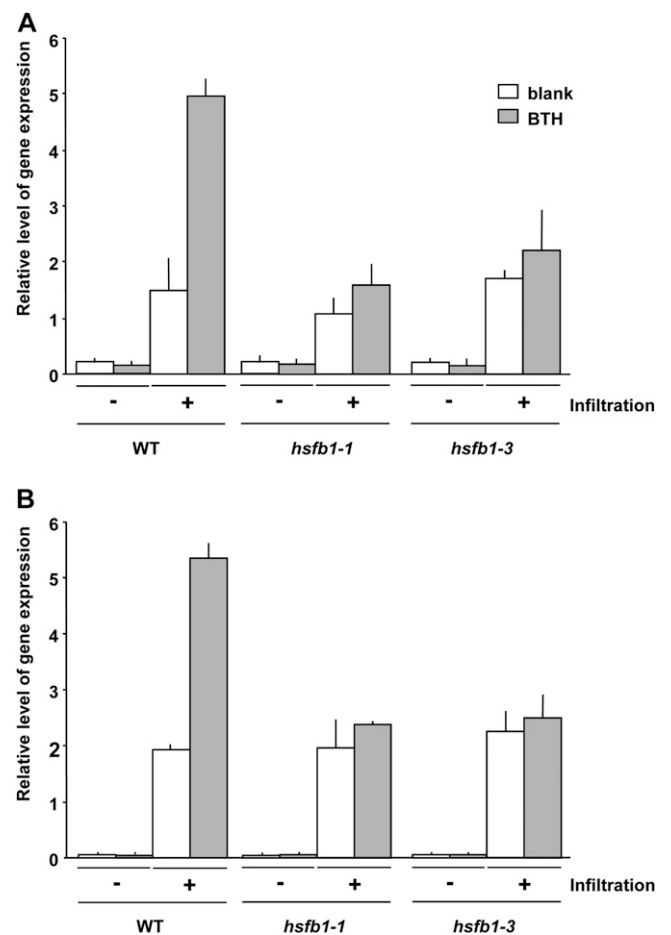

Figure 2. Absence of primed defense gene activation in *hsfb1* mutants. Five-week-old wild-type (WT), *hsfb1-1*, and *hsfb1-3* plants were grown as described in Figure 1 and treated with 100 μM BTH or the blank formulation for 3 d. Then, leaves were either left untreated (−) or infiltrated with water (+). Three hours later, leaves were assayed for the accumulation of *PAL1* (A) or *WRKY29* (B) transcripts by qRT-PCR. Relative amounts of gene expression were normalized to those of *ACTIN2*. Error bars indicate SD (*n* = 3). The experiment was repeated three times with similar results.
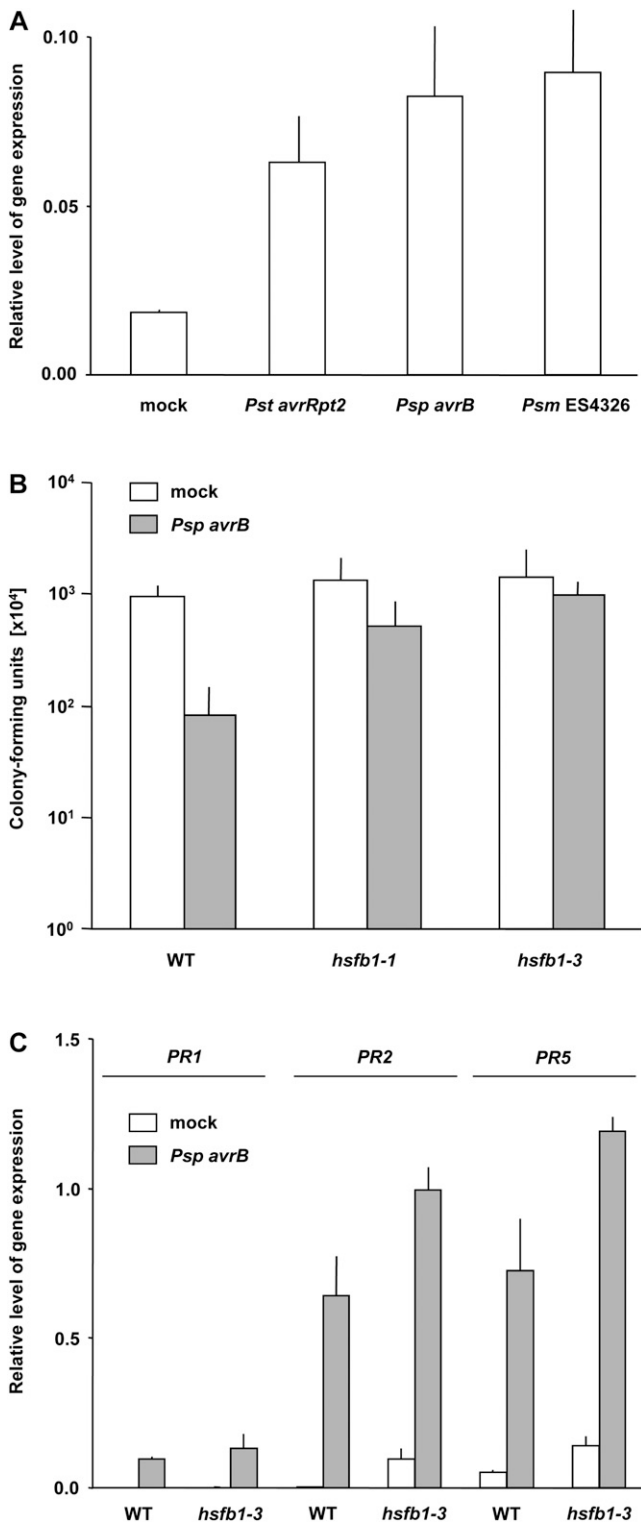
Figure 3. Bacteria-induced gene expression and bacterial multiplication in systemic leaves of primary inoculated plants. A, Five-week-old Arabidopsis wild-type plants were pressure infiltrated on four lower leaves with suspensions of *Pst avrRpt2* (optical density at 600 nm [OD$_{600}$] = 0.002), *Psm* ES4326 (OD$_{600}$ = 0.002), or *Psp avrB* (OD$_{600}$ = 0.002) in 10 mM MgCl$_2$. Control plants were infiltrated with 10 mM MgCl$_2$ in the absence of bacteria (mock). After 3 d, systemic leaves

(Pajerowska-Mukhtar et al., 2012), and primed defense gene expression (Fig. 2), might also be pivotal for pathogen-induced, bona fide SAR. Arabidopsis plants were inoculated on three lower leaves with SAR-inducing avirulent *Pseudomonas syringae* pv *tomato* (*Pst*) DC3000 harboring the avirulence gene avrRpt2 (*Pst avrRpt2*), *Pseudomonas syringae* pv *phaseolicola* (*Psp*) carrying *avrB* (*Psp avrB*), or *Pseudomonas syringae* pv *maculicola* (*Psm*) strain ES4326 (*Psm* ES4326). *Pst avrRpt2* and *Psp avrB* are avirulent to the Columbia-0 accession of Arabidopsis and trigger a hypersensitive response (Whalen et al., 1991; Gopalan et al., 1996), whereas *Psm* ES4326 is virulent to Columbia-0 plants (Dong et al., 1991). Three days after primary inoculation with any of these three bacterial strains, systemic leaves were harvested and assayed for the accumulation of *HsfB1* transcripts. As depicted in Figure 3A, the three SAR-inducing bacterial strains activate the expression of *HsfB1* in systemic leaves of locally infected Arabidopsis plants. This finding suggested that HsfB1 indeed could be an important component of bacteria-induced SAR in Arabidopsis.

To test whether this is the case, wild-type, *hsfb1-1*, and *hsfb1-3* plants were inoculated on three lower leaves with *Psp avrB*. After 3 d, systemic leaves were challenge inoculated with *Psm* ES4326. After another 3 d, *Psm* ES4326-infected systemic leaves were assayed for bacterial multiplication. As shown in Figure 3B, the primary inoculation of wild-type plants with *Psp avrB* reduced the number of *Psm* ES4326 bacteria reisolated from systemic leaves about 10-fold. This finding verified the presence of SAR in *Psp avrB*-inoculated wild-type plants. In contrast, the reduction in bacterial counts reisolated from systemic leaves of the primary inoculated *hsfb1-1* and *hsfb1-3* mutants was much lower and did not show significant differences from bacterial multiplication in mock-inoculated wild-type or *hsfb1* mutant plants (Fig. 3B). These findings demonstrated that HsfB1 is a critical component in *Psp avrB*-induced SAR to *Psm* ES4326 in Arabidopsis. Because localized infection with *Psp avrB* did not significantly affect (*PR1*) or even enhance (*PR2* and *PR5*) the

activation of the SAR marker genes *PR1*, *PR2*, and *PR5* in systemic leaves of the *hsfb1-3* mutant (Fig. 3C), HsfB1 seems to be associated with the priming and not simply the activation of defense genes during SAR. This result also indicates that systemic signaling is not affected in *hsfb1-3*. Also, as wild-type and *hsfb1-3* plants in the absence of previous SAR activation did not show differences in supporting bacterial growth after primary infection with virulent *Psm* ES4326 or avirulent *Psp avrB* (Supplemental Fig. S2), the perception of and response to these two bacterial strains per se seem not to be affected in *hsfb1-3*.

CONCLUSION

It was shown recently that HsfB1 is a crucial component of acquired thermotolerance and salicylic acid-mediated resistance in Arabidopsis. We complemented these findings by demonstrating that HsfB1 plays a pivotal role also in primed defense gene activation and pathogen-induced SAR in this plant. Together, these data disclose HsfB1 as a common key player in acquired tolerance to both biotic and abiotic stresses. These findings also provide new knowledge on the molecular mechanism of defense priming in plants.

The Arabidopsis Information Resource stock names for *hsfb1-1* and *hsfb1-3* are SALK_104713 and SALK_106223.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Analysis of T-DNA-tagged *hsfB1-1* and *hsfB1-3* lines.

Supplemental Figure S2. Multiplication of *Psm* ES4326 and *Psp avrB* in the wild type and the *hsfb1-3* mutant.

Supplemental Table S1. List of gene-specific forward and reverse primers used for real-time qRT-PCR analysis.

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