LOSS OF CALMODULIN BINDING TO BAX INHIBITOR-1 AFFECTS PSEUDOMONAS MEDIATED HYPERSENSITIVE RESPONSE-CELL DEATH IN ARABIDOPSIS THALIANA

Maki Kawai-Yamada1,3,4, Zenta Hori3, Taro Ogawa3, Yuri Ihara-Ohori3, Katsunori Tamura3, Minoru Nagano3, Toshiki Ishikawa1,3,4 and Hirofumi Uchimiya2,3

1Department of Environmental Science and Technology, Saitama University, 255 Shimo-Okubo, Sakura-ku, Saitama 338-8570, Japan
2Iwate Biotechnology Research Center, Kitakami, Iwate 024-0003, Japan
3Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan
4Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST), Japan

Address corresponding to: Maki Kawai-Yamada, PhD., Department of Environmental Science and Technology, Saitama University, 255 Shimo-Okubo, Sakura-ku, Saitama 338-8570, Japan. Fax & Tel: +81-48-858-9269. Email: mkawai@mail.saitama-u.ac.jp

Bax inhibitor-1 (BI-1) is a cell-death suppressor protein conserved across a variety of organisms. The Arabidopsis atbi1-1 plant is a mutant, in which the C-terminal 6 amino acids of the expressed BI-1 protein have been replaced by T-DNA insertion. This mutant BI-1 protein (AtBI-CM) produced in E. coli can no longer bind to calmodulin. A promoter-reporter assay demonstrated compartmented expression of BI-1 during hypersensitive response (HR), introduced by the inoculation of Pseudomonas syringae possessing the avrRTP2 gene, Pst(avnRTP2). In addition, both BI-1 knockdown plants and atbi1-1 showed increased sensitivity to Pst(avnRTP2)-induced cell death. The results indicated that the loss of calmodulin binding reduces the cell-death suppressor activity of BI-1 in planta.
Experimental Procedures

Plant materials
The Columbia (Col-0) ecotype of *Arabidopsis thaliana* (L.) Heynh was used as the wild-type plant in this study. Seeds of *Arabidopsis* were sterilized and either inoculated onto plates containing 0.2% Gerangam, 2% (w/v) sucrose, and 0.5 x MS medium or grown on a Jiffy-7 (Jiffy Product International AS, Norway). All plant material was grown at 23°C under continuous light conditions (60 µmol m-2s-1).

AtBI-1 knockdown plant (AtBI-RNAi strains) and GFP containing control plant were described previously (5). The T-DNA-inserted line, *atbi1-1*, was obtained from Syngenta as Garlic_228_D08, and T-DNA insertion was confirmed by PCR and sequence determination.

Yeast experiments
*Saccharomyces cerevisiae* wild-type strain W303-1A was used in the present study. The Yep51-Bax expression vector was provided by Dr. Reed (22). The pYX112-AtBI-CM expression vector was constructed by using the PCR-amplified EcoRI-tagged *atbi1-1* cDNA fragment. The pYX112-AtBI-1 vector was described previously (23,26). Yeast strains carrying Yep51-Bax were transformed with pYX112, pYX112-AtBI-1, or pYX112-AtBI-CM by the lithium acetate method. Yeast strains were cultured in YPD (1% yeast extract, 2% peptone, and 2% glucose) or synthetic dextrose (SD) medium with appropriate supplements at 30°C. Ura-Leu' transformants were streaked either on SD-glucose (Glc) or on SD-galactose (Gal) plates, and incubated at 30°C for 3 (Glc) or 4 (Gal) days, respectively.

Total yeast RNA was extracted using the RNeasy plant miniprep kit (Qiagen, Hilden, Germany). Purified RNA (0.2 µg) was used for RT-PCR using specific primers for *AtBI-1*: a, 5’-ATGAGCATCCTTATCACTGCATT-3’; b, 5’-CTTCAACATTATGATGAGAAT-3’; c, 5’-GTTTCTCCTTTTCTTCTC-3’; d, 5’-CACCACAAAGCGGTACCACTTCTTCT-3’. PCR reactions were performed in 10 µl using a direct amplification system (Ready to Go RT-PCR beads, Amersham Pharmacia Biotech, Piscataway, NJ) as described in Oshima et al. (24).

Overlay Assay
The interaction of AtBI-1 with calmodulin was studied by overlay assay basically as described previously (5). The C-terminal 14 amino acids of AtBI-1, AtBI-CM, AtBI-29, and AtBI-30 were ligated into the pMAL vector (NEB) to express MBP-tagged proteins. The resultant plasmids were transformed into *E. coli* BL21 strain and MBP-tagged C-terminal proteins were purified according to the instructions provided by the manufacturer (NEB). The empty pMAL vector carrying in-frame β-galactosidase protein was used as a control (Gal). The His- and S-tagged AtCaM7 protein was also purified as described previously (5). The purified C-terminal and Gal (control) proteins were subjected to SDS-PAGE, blotted onto PVDF membrane, and immunodetected with anti-MBP antibody (NEB) as a control for protein loading. S-tagged AtCaM7 protein in 1 x phosphate-buffered saline (PBS) with 1 mM CaCl₂ was used as the overlay. Binding was detected using horseradish peroxidase-conjugated S-protein (Novagen, Tokyo) and visualized by chemiluminescence (ECL kit, Amersham Biosciences). The coding region of *Arabidopsis* calmodulin and calmodulin-related proteins (AtCaM3, AtCaM6, AtCaM8, AtCaM9, AtCML23, and AtCML12) were also amplified by PCR, ligated into the pMAL vector, and used in this assay. Primers used for PCR were as follows: AtCaM3, 5’-CACCATGGGCGGAT CAGCTCACC-3’ and 5’-CTTCCATCATC-3’; AtCaM6, 5’-CACCATGGCGGATCAGCTAAC-3’ and 5’-CTTGCACC ATCATGACTTTG-3’; AtCaM8, 5’-CACCATGGCGGATCAGCTAAC-3’ and 5’-CACCATGGCGGATCAGCTAAC-3’; AtCaM9, 5’-CACCATGGCGGATCAGCTAAC-3’ and 5’-CACCATGGCGGATCAGCTAAC-3’; AtCaM12, 5’-CACCATGGCGGATCAGCTAAC-3’ and 5’-CACCATGGCGGATCAGCTAAC-3’.

GUS assay and microscopic analysis
The promoter-GUS reporter assay plasmid consisted of 2 kbp of *AtBI-1* promoter region and the N-terminal 10 amino acids of AtBI-1 fused with the GUS reporter. The genomic fragment of *AtBI-1* was amplified with 5’-GGTCGAC CAAGTCAAAGACCTTCGATACAT-3’ and 5’-TCTAGACCAGCTTCTGCCAG-3’ and 5’-CTTCAACATTATGATGAGAAT-3’; c, 5’-GTTTCTCCTTTTCTTCTC-3’; PCR reactions were performed in 10 µl using a direct amplification system (Ready to Go RT-PCR beads, Amersham Pharmacia Biotech, Piscataway, NJ) as described in Oshima et al. (24).
plants were obtained by Agrobacterium-mediated transformation. More than 10 of transgenic Arabidopsis lines possessing AtBI-1 – promoter -GUS construct were obtained. Among them, three lines, which show stable and typical GUS staining patterns were chosen for further study. For the GUS assay, plant tissues were fixed in 90% acetone for 10 min on ice, and vacuum infiltrated with X-gluc buffer [0.1 M NaPO₄ buffer, pH 7.0, 10 mM ethylenediaminetetraacetic acid (EDTA), 3 mM potassium ferrocyanide, 3 mM potassium ferrocyanide, 0.1% [v/v] Triton X-100, and 0.5 mg/mL X-gluc]. Leaves incubated overnight in the dark at 37°C were decolored with acetic acid:ethanol (1:6) solution at room temperature. Transparentized tissues treated with chloride hydrate were observed and photographed. Autofluorescence induced by bacterial infection was monitored and imaged by fluorescence microscopy (MMRD; Leica, Wetzlar, Germany).

Infection with Pseudomonas
Pseudomonas syringae pv tomato DC3000 strains containing avrRpt2, Pst(avrRpt2), or the empty plasmid vector Pst(vec) were grown for 1 day on King’s B medium containing appropriate antibiotics (25 µg/mL rifampicin and 25 µg/mL kanamycin) at 30°C. Leaves were inoculated with 5 x 10⁸ cfu/ml of P. syringae in 10 mM MgCl₂, using 1-ml plastic syringes without needles. To detect ion leakage, three leaf discs obtained from plants inoculated with Pseudomonas were floated on autoclaved distilled water. Electrolyte leakage was measured using an electrical conductivity meter (B-173; Horiba, Kyoto, Japan) at several time points, and expressed as a relative value against total ion leakage, which was measured in the autoclaved samples.

Bacterial growth was measured at 4 day-after infiltration by extracting bacteria from leaf discs and plating a series of dilutions on the King’s B medium supplemented with appropriate antibiotics.

RESULTS

Requirement of BI-1 C-terminus for calmodulin binding
The AtBI-1 gene (At5G47120) consists of 5 introns and 6 exons. As reported previously, one of the T-DNA-tagged Arabidopsis lines, atbi-1 (SAIL_228_D08), has a T-DNA insertion in exon 6. The atbi-1 plants show accelerated progression of cell death upon infiltration of leaf with fungal toxin FB1, as well as increased sensitivity to heat-shock- and tunicamycin-induced cell death (6,7). We also independently obtained the T-DNA-inserted plant (Garlic_228_D08, Syngenta), equivalent to SAIL_228_D08, and confirmed that this plant expresses mRNA containing the inserted boundary sequence of T-DNA encoding 6 replacement amino acids in-frame at the C-terminus (Fig. 1). In addition, the plant showed no specific phenotype under normal growth conditions, similar to the AtBI-RNAi plant, in which AtBI-1 was downregulated by RNAi method (Fig. 1B)(5).

To test if the atbi-1 C-terminal mutant protein functions normally as a cell-death suppressor, we assayed for rescue of Bax-induced yeast lethality. As reported previously, a C-terminal variant with a high coiled-coil score, AtBI-29, retained cell-death suppression activity, whereas the AtBI-30 mutant with a reduced coiled-coil score failed to suppress Bax-induced lethality in yeast (Fig. 1C)(5). Another C-terminal variant, AtBI-CM (resultant product of atbi-1), had a coiled-coil score of 0.01 (Fig. 1C). As demonstrated in Fig. 2A, the yeast strain containing galactose-inducible proapoptotic protein Bax (Bax+vec) showed growth suppression on galactose-containing medium (Gal). Yeast cells expressing wild-type AtBI-1 recovered their growth activity on the Gal medium, whereas the AtBI-CM protein failed to suppress Bax-induced lethality. The expression of AtBI-1 and AtBI-CM mRNAs was confirmed by RT-PCR (Fig. 2B).

The C-terminal 14-residue domain of AtBI-1 interacts with calmodulin (5). Overlay binding assays were used to analyze whether calmodulin binding is coincident with the suppressor function of AtBI-1. Fusion proteins of maltose binding protein (MBP) and the C-terminus of AtBI-1 is in agreement with previous work, AtBI-1 interacted with AtCaM7. In addition, AtBI-29, which retained its cell-death suppression activity, interacted with AtCaM7, whereas AtBI-30 and AtBI-CM showed reduced binding with calmodulin. These results suggest that calmodulin binding to the C-terminal region of AtBI-1 is in agreement with the suppressor function of the protein in yeast.

Plant species have evolved complex and intricate calcium signaling cascades. For instance, database searching predicts more than 50 of calmodulin-related proteins (CaMs and CMLs) encoded by the Arabidopsis genome. To
define the calmodulin molecules that interact with AtBI-1, several classes of these proteins were expressed in *E. coli*, purified, and analyzed by overlay assay (Fig. 4). Only three of the calmodulins tested (CaM3, CaM6, and CaM7) interacted with AtBI-1, and all of these belong to the authentic calmodulin family gene group, which is highly conserved across a range of organisms.

*AtBI-1 is associated with the pathogen response*

Rice cells overexpressing AtBI-1 show resistance to the HR-like cell death induced by an elicitor derived from *Magnaporthe grisea* (3). To further characterize the tissue specificity of AtBI-1 expression, a GUS reporter gene was cloned under the control of the AtBI-1 promoter region (-2 kbp). After the successive transformation, we obtained more than 10 of single GUS gene-inserted lines. Among them, three lines, which show stable and typical GUS staining patterns were chosen for further study. Whole-mount GUS staining demonstrated high expression levels in young leaves and roots of two-week-old seedlings (Fig. 5A). The highest expression in roots was seen at the root tip and in vascular tissue (Fig. 5B). Strong staining was also found in flower tissues, including stamens and sepals, and in the base of siliques (Fig. 5D, E-J). In contrast, a mature leaf did not show clear GUS activity (Fig. 5C). Thus the mature leaves (3-5th leaves of 3 week old plants) were used for followed experiments.

Although *AtBI-1* gene expression is known to be stress-responsive (9), the tissue specificity is not known. To evaluate *AtBI-1* expression at the tissue level, mature leaves of transgenic plants containing the GUS reporter gene under the control of an *AtBI-1* promoter were infected with *Pseudomonas* containing either empty vector or *avirulent Rpt2* (10). As shown in Fig. 6A, site-specific GUS expression was observed in leaves inoculated for 16 h with Pst(*avrRpt2*), whereas mock-inoculated (Mock) and vector (Pst(vec)) controls showed only non-tissue-specific expression of a lower magnitude. Leaf cells that underwent HR demonstrated autofluorescence, and as shown in Fig. 6B-D, the GUS activity was largely coincident with autofluorescence. Furthermore, *Arabidopsis* plants that exhibited altered *AtBI-1* expression (overexpressed line, knockdown, and C-terminal mutant) were inoculated with *P. syringae* expressing *avrRpt2*. Electrolyte leakage, measured as an increase in conductivity of the solution containing the treated leaf discs, is often used to assay irreversible membrane damage during HR (11). No clear leakage was detected during the first 3 h of incubation (Fig. 6E), but 6 h after inoculation, the C-terminal mutant (*CM/atbi1-1*) and knockdown (KD) lines exhibited elevated sensitivity to HR cell death. We also assessed the bacterial growth of Pst(*avrRpt2*) in infected leaves. The C-terminal mutant (*CM/ atbi1-1*) and RNAi (KD) plants demonstrated reduced bacterial growth, suggesting that AtBI-1 is associated with Pst(*avrRpt2*)-triggered immunity.

**DISCUSSION**

The *Arabidopsis* genome harbors seven calmodulins and about 50 of calmodulin-like genes (12,27). In spite of their potential importance in mediating plant calcium signaling, the physiological functions of these calmodulin-related genes remain largely unknown due to redundancy. Only seven of the *Arabidopsis* calmodulin genes encode proteins with high identity to vertebrate calmodulins. These belong to a class of plant calmodulins that activates NAD kinase in vitro and binds both kinesin-like motor proteins and cyclic nucleotide-gated ion channels (13-15). We have demonstrated that the interaction between BI-1 and calmodulin using a split ubiquitin two-hybrid system, and similar binding in plant cells by BiFC analysis (5). Calmodulin binds to the C-terminal cytoplasmic tail of BI-1, which is relatively conserved among BI-1 family members. In this study, we demonstrated that genomic BI-1 mutant (*AtBI-CM*) lacked the calmodulin binding activity, which in turn caused increased sensitivity to Pst(*avrRpt2*). Overlay assays performed in this study to determine which type of calmodulin-related protein binds BI-1 in vitro demonstrated that the authentic conserved type of calmodulins binds to the C-terminus of AtBI-1. Microarray expression data which is available in Web site made us to estimate which calmodulin-related molecule is co-expressed with *AtBI-1* (See supplemental data). The *AtBI-1*, CaMs and CMLs were expressed in almost tissues at least in basal level, and did not show typical tissue specificity. However, the expression data in response to different stresses revealed distinct patterns of co-expression. Interestingly, the CaM mRNA co-expressed with *AtBI-1* during the response to Pst(*avrRPT2*) was At3g43810 (CaM7), which is the first identified ABI-1 binding protein reported in our previous study (5). In contrast, CaMs co-expressing under abiotic stresses (cold, osmotic, UV-B, wounding and heat) were
At3g56800 (CaM3) and At2g41110 (CaM2). These data are in agreement with the present study, that AtBI-1 binds authentic CaM proteins, not CML molecules. To clarify the functional partner for AtBI-1 under each stress condition, further analysis using single or multi gene disruptions of CaM will be useful. The basic structure of Arabidopsis calmodulins is highly similar to those of animal calmodulins in both the Ca$^{2+}$-binding loops and EF hands. Thus, it would be also interesting to establish whether the function of mammalian BI-1 also requires calmodulin binding.

Cytosolic calcium increases in response to a range of diverse stimuli, including environmental, hormonal and developmental prompts. This elevation is sensed by calmodulin and transduced into altered target activity to trigger a cellular response. Calcium plays a major role in initiating the HR, which in turn limits pathogen growth by promoting plant cell wall reinforcement, or by eliminating host functions necessary for pathogen multiplication (16). The HR of plants to avirulent pathogens effectively restricts pathogen growth and includes a characteristic programmed cell death at the site of pathogen invasion (17). Such events typically include the induction of reactive oxygen species (ROS), callose deposition, and expression of several defense-related genes (18). This study focused on HR mediated by the Arabidopsis R gene RPS2 to infection by the bacterial pathogen P. syringae pv tomato DC3000 (Pst) carrying the type III effector gene avrRpt2. The type III effector was first identified based on its ability to trigger pathogen recognition in resistant host plants (19), and interactions between the products of the avrRpt2 gene and the RPS2 resistance gene is a well characterized example of HR-induced cell death (20). Sanchez et al. (21) demonstrated that AtBI-1 gene expression is rapidly upregulated during wounding or pathogen challenge. A reporter gene linked to the promoter from AtBI-1 provided direct evidence that the infection by Pseudomonas possessing the avrRpt2 gene can regulate AtBI-1 transcription, with the GUS signal surrounding the lesioned region. The ion leakage occurs at the late stage of HR. The loss of plasma membrane semipermeability and the protoplast shrinkage causes ion leak (29). Cells dying via the HR usually become autofluorescent and brown, attributed to accumulation of phenolic compounds (30). The autofluorescence is observed in both, cell wall of dying cells and adjacent living cells in close contact with the infected site. Many defense genes are induced with the dying cells and in their adjacent living neighbors (29). Thus, the HR associates with both cell death and defense. The expression of AtBI-1 in the adjacent neighbor of infected region may suggest the role of BI-1 in defense response with unknown function. Recently, we reported that cytochrome b5, which is an electron transfer protein in lipid metabolism in ER is another AtBI-1 binding protein in Arabidopsis (31). One possible speculation is that AtBI-1 would be associated with membrane re-modification of adjacent neighbor of infected site.

Regarding AtBI-1 overexpressing plants, we did not see any phenotypes on the ion leakage or bacterial growth (data not shown). This is an agreement with the previous studies (6). AtBI-1 mutant showed higher sensitivity against FB1 or heat shock treatment, however the overexpression line did not show any differences. We assume that the native level of AtBI-1 expression may be sufficient or saturated to execute its function as a cell death suppressor in wild type Arabidopsis plants.

Elucidating the biological function of BI-1 protein is a significant future challenge for the field of plant cell death regulation. For one thing, it would help to clarify the cell protection mechanism operating in plants. Furthermore, understanding the relationship between BI-1 and calmodulin might reveal how the versatile nature of calcium signaling facilitates the dynamic behaviors and environmental adaptability typical of plants.
REFERENCES

FOOTNOTES
Key words: Bax inhibitor-1, calmodulin, cell death, hypersensitive response
Abbreviations: BI; Bax Inhibitor, CaM; calmodulin, HR; hypersensitive response, MBP; maltose binding protein

Acknowledgment
We thank Dr. J.C. Reed for kindly providing the described material. We also thank Ms. Y. Takahashi and Ms. M. Sakamoto for technical assistance. This work was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan, and a grant from CREST, JST, Japan.

FIGURE LEGENDS

Fig. 1. Schematic diagram of AtBI-1 mutants used in this study.  
(A) Genetic structure of the AtBI-1 and T-DNA-inserted mutant (AtBI-CM/atbi1-1). Exons are represented by numbered open boxes and introns by lines. (B) Representative phenotype of atbi1-1 and AtBI-RNAi plant. Plants were grown for 3 weeks under continuous light at 22°C. (C) AtBI-1 mutants analyzed in the present study. The C-terminal 6 amino acids were replaced in mutant proteins (AtBI-CM, AtBI-29 and AtBI-30). Replaced amino acids are underlined. Calculated scores for coiled-coil structure (available at http://www.ch.embnet.org/software/COILS_form.html) and cell-death suppression activity in yeast analyzed previously (5,8) are indicated.

Fig. 2. AtBI-CM failed to suppress Bax-induced lethality in yeast cells.  
(A) Suppression of Bax-induced yeast lethality by AtBI-1 and AtBI-CM. Yeast cells possessing the galactose-inducible Bax gene with pYX112 (Bax+Vec), pYX112-AtBI-1 (Bax+AtBI-1), or pYX112-AtBI-CM (Bax+AtBI-CM) were streaked on glucose (Glc)- or galactose (Gal)-containing medium. (B) RT-PCR analysis of AtBI-1 expression in yeast strains possessing pYX112 (Vec), pYX112-AtBI-1 (AtBI-1), and pYX112-AtBI-CM (AtBI-CM). Exon numbers and the positions of the oligonucleotides (a-d) used for RT-PCR are indicated (upper panel). Ethidium bromide staining of rRNAs served as loading controls.

Fig. 3. Interaction of AtBI-1 C-terminal mutants with Arabidopsis calmodulin 7 (AtCaM7). The purified MBP-tagged C-terminal fragments of AtBI-1, AtBI-29, AtBI-30, AtBI-CM, and β-galactosidase (Gal, produced by empty vector) were separated by SDS-PAGE, immunoblotted, and examined by overlay assay. Coomassie Brilliant Blue staining (CBB), western blotting with anti-MBP antibody, and overlay assay are shown.

Fig. 4. AtBI-1 interacts with authentic CaM proteins.  
(A) Arabidopsis calmodulin and calmodulin-related proteins (AtCaM3, AtCaM6, AtCaM7, AtCaM8, AtCaM9, AtCML23, and AtCML12) fused with His- and S-tags were expressed in E. coli, and purified proteins were used for the overlay assay with AtBI-1. CBB staining (CBB) and western blotting with anti-MBP antibody (anti-MBP) were also examined as controls for protein loading. Molecular size marker (kDa) are indicated on the left. (B) Phylogenic relationships of Arabidopsis calmodulin-related proteins and interaction with AtBI-1. A phylogenic tree of CaMs and selected CMLs was constructed by the CLUSTALW program. Proteins analyzed in this study are denoted by boxes with the identified interaction (+ or -). The designation of genes was in accordance with McCormack et al (12).

Fig. 5. Histochemical analysis of GUS expression in AtBI-1 promoter-GUS transgenic plants. The GUS reporter gene was expressed in wild-type Arabidopsis plants under the control of the AtBI-1 promoter region (2 kbp). (A) Young seedling (12 day-old). (B) Root. (C) Mature leaf (4th leaf in 3 week-old). (D) Flowers of control plant without transgene. (E and F) Flower buds of GUS-containing plant. (G) Magnified flower buds. (H) Blooming flower. (I) Stamen and anther. (J) Silique. Bar = 1 mm.
Fig. 6. AtBI-1 expression associated with HR caused by Pseudomonas syringae pv tomato DC3000 carrying avrRpt2. (A) Histochemical expression analysis of AtBI promoter-GUS in HR caused by Pst(avrRpt2) inoculation. The 3-5th leaves of Arabidopsis plants carrying the AtBI-promoter-GUS construct were inoculated with 10 mM MgCl₂ (Mock), Pst(vec), or Pst(avrRpt2). After 1 day, leaves were stained for GUS activity. (B) Magnified image of leaf inoculated with Pst(avrRpt2). Asterisks indicate the inoculated sites. (C and D) Magnified view of boxed region in (B) and autofluorescence observed by fluorescence microscopy, respectively. Bars = 1 mm. (E) Electrolyte leakage induced by HR cell death. The electrical conductivity was measured in the Pst(avrRPT2)-inoculated control (GFP), AtBI-1 knockdown (KD), and atbi1-1 (CM) plants as described in Materials and Methods. Data are mean±SD of 5 plants each. (F) Bacterial growth in control (GFP) and C-terminal mutant. Arabidopsis plants (GFP, CM, and KD) were inoculated with Pst(avrRpt2) and bacterial growth was measured at 4 day-after inoculation. *Significant difference (P<0.05) between the control (GFP) and CM or KD (n=5).

Supporting Information
Figure S1. Hierarchical clustering of AtBI-1 and calmodulin (CAM)/calmodulin-like proteins (CML) according to expression patterns during avirulent P. syringae (avrRpt2) infection (A) and various abiotic stresses (cold, osmotic, UV-B, wounding and heat stresses) (B). Expression data obtained from the Arabidopsis Electronic Fluorescent Pictograph (eFP) browser (28) were normalized and subjected to hierarchical cluster analysis and dendrogram generation using the algorithms embedded in the R software package. Heat map shows the normalized values with the color scale.
Fig. 1
Kawai-Yamada et al
Fig. 2
Kawai-Yamada et al
Fig. 4
Kawai-Yamada et al
Fig. 5
Kawai-Yamada et al
Loss of calmodulin binding to Bax inhibitor-1 affects Pseudomonas-mediated hypersensitive response-cell death in Arabidopsis thaliana

Maki Kawai-Yamada, Zenta Hori, Taro Ogawa, Yuri Ihara-Ohori, Katsunori Tamura, Minoru Nagano, Toshiki Ishikawa and Hirofumi Uchimiya

J. Biol. Chem. published online August 12, 2009

Access the most updated version of this article at doi: 10.1074/jbc.M109.037234

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2009/08/12/M109.037234.DC1

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/early/2009/08/12/jbc.M109.037234.full.html#ref-list-1