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The *Arabidopsis* Leucine-Rich Repeat Receptor–Like Kinases BAK1/SERK3 and BKK1/SERK4 Are Required for Innate Immunity to Hemibiotrophic and Biotrophic Pathogens [W]

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Recognition of pathogen-associated molecular patterns (PAMPs) by surface-localized pattern recognition receptors (PRRs) constitutes an important layer of innate immunity in plants. The leucine-rich repeat (LRR) receptor kinases EF-TU RECEPTOR (EFR) and FLAGELLIN SENSING2 (FLS2) are the PRRs for the peptide PAMPs elf18 and flg22, which are derived from bacterial EF-Tu and flagellin, respectively. Using coimmunoprecipitation and mass spectrometry analyses, we demonstrated that EFR and FLS2 undergo ligand-induced heteromerization in planta with several LRR receptor-like kinases that belong to the SOMATIC-EMBRYOGENESIS RECEPTOR-LIKE KINASE (SERK) family, including BRASSINOSTEROID INSENSITIVE1-ASSOCIATED KINASE1/SERK3 (BAK1/SERK3) and BAK1-LIKE1/SERK4 (BKK1/SERK4). Using a novel *bak1* allele that does not exhibit pleiotropic defects in brassinosteroid and cell death responses, we determined that BAK1 and BKK1 cooperate genetically to achieve full signaling capability in response to elf18 and flg22 and to the damage-associated molecular pattern AtPep1. Furthermore, we demonstrated that BAK1 and BKK1 contribute to disease resistance against the hemibiotrophic bacterium *Pseudomonas syringae* and the obligate biotrophic oomycete *Hyaloperonospora arabidopsidis*. Our work reveals that the establishment of PAMP-triggered immunity (PTI) relies on the rapid ligand-induced recruitment of multiple SERKs within PRR complexes and provides insight into the early PTI signaling events underlying this important layer of plant innate immunity.

INTRODUCTION

Plants defend themselves against pathogenic organisms by detecting potential invaders using a sensitive, multilayered innate immune system. The primary layer of inducible defense relies on the detection of conserved microbial molecules that act as signatures of a whole class of microbes. These molecules are referred to as pathogen-associated molecular patterns (PAMPs), most of which are perceived by transmembrane pattern recognition receptors (PRRs) (Zipfel, 2008; Boller and Felix, 2009). PAMP perception leads to a series of downstream events, including ion fluxes, production of reactive oxygen species (ROS) in an oxidative burst, activation of mitogen-activated protein (MAP) kinases and calcium-dependent protein kinases, induction of defense gene expression, and callose deposition (Nicaise et al., 2009; Boudsocq et al., 2010). All of these outputs culminate in PAMP-triggered immunity (PTI) that confers resistance to a broad range of pathogens (Jones and Dangl, 2006; Boller and Felix, 2009).

In *Arabidopsis thaliana*, the best-studied PRRs are the leucine-rich repeat receptor kinases (LRR-RKs) FLAGELLIN SENSING2 (FLS2) and EF-TU RECEPTOR (EFR) that specifically bind the bacterial peptide PAMPs flg22 (derived from flagellin) and elf18/elf26 (derived from elongation factor Tu), respectively (Chinchilla et al., 2006; Zipfel et al., 2006). Both FLS2 and EFR are critical for antibacterial immunity. Plants affected in flagellin perception are more susceptible to adapted and nonadapted *Pseudomonas syringae* strains (Zipfel, 2009), and loss of EFR leads to enhanced susceptibility to *Agrobacterium tumefaciens* and to weakly virulent strains of *P. syringae* pv *tomato* DC3000 (*Pto* DC3000) (Nekrasov et al., 2009; Zipfel, 2009). Notably, heterologous expression of *Arabidopsis EFR* in *Nicotiana benthamiana* and tomato (*Solanum lycopersicum*) plants increases their resistance to several highly virulent bacterial pathogens (Lacombe et al., 2010).

In addition to PAMPs, PRRs can also perceive other specific signals, referred to as damage-associated molecular patterns (DAMPs) (Boller and Felix, 2009; Tör et al., 2009). These endogenous molecules are normally not available for recognition but

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are released or produced under stress conditions. For example, the LRR-RKs PEP RECEPTOR1 (PEPR1) and PEPR2 recognize the PAMP- and wound-induced endogenous peptide Pep1 and its paralogs that are thought to act as amplifiers of PTI signaling (Yamaguchi et al., 2006, 2010; Huffaker and Ryan, 2007; Krol et al., 2010).

Despite the importance of PRRs in plant innate immunity, the exact molecular events immediately following PAMP perception are poorly understood. RKs possess all necessary domains to bind extracellular ligands and to relay perception events into cytoplasmic signaling cascades. However, paradigmatic models based on animal receptor Tyr kinases show that ligand binding RKs form homo- and heterooligomeric complexes with additional RKs underlying transphosphorylation events (Lemmon and Schlessinger, 2010). A similar example is provided in plants by the LRR-RK brassinosteroid (BR) receptor BRASSINOSTEROID INSENSITIVE1 (BRI1) that forms a ligand-inducible complex with the LRR-RK BRI1-ASSOCIATED RECEPTOR-LIKE KINASE/SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE3 (BAK1/SERK3) (Li et al., 2002; Nam and Li, 2002; Wang et al., 2005). The BRI1-BAK1 heteromerization results in sequential reciprocal receptor transphosphorylation, which ultimately increases the kinase activity of BRI1 to enhance downstream signaling outputs (Wang et al., 2008). BAK1 acts as a positive regulator of the BRI1 pathway, as null *bak1* mutants are hyposensitive to BR. Unexpectedly, BAK1 was recently found to also form a ligand-dependent complex with FLS2 (Chinchilla et al., 2007; Heese et al., 2007). This association occurs within seconds of flg22 binding and leads to rapid phosphorylation of FLS2 and BAK1 (Schulze et al., 2010). Loss of *BAK1* results in reduced flg22 responses (Chinchilla et al., 2007; Heese et al., 2007). BAK1 is also required for responses triggered by the bacterial PAMPs elf18, lipopolysaccharides (LPSs), peptidoglycans (PGNs), HrpZ, csp22 (derived from cold shock protein), the oomycete PAMP INF1, and the DAMP AtPep1 (Chinchilla et al., 2007; Heese et al., 2007; Shan et al., 2008; Krol et al., 2010), suggesting that BAK1 may also form a ligand-dependent complex with their corresponding PRRs. PEPR1 and PEPR2 have been identified recently as BAK1-interacting proteins in a targeted yeast two-hybrid approach (Postel et al., 2010). Interestingly, elf26 treatment leads to rapid phosphorylation of BAK1 and of a coimmunoprecipitated protein that migrates at the same size as the glycosylated form of EFR (Schulze et al., 2010). Notably, the effect of *BAK1* loss of function on elf18 responses is less marked than for flg22 responses (Chinchilla et al., 2007; Shan et al., 2008), and null *bak1* mutant plants are still sensitive to flg22 and other PAMPs. This indicates that EFR may preferentially interact with other RLKs than BAK1 and that additional complex components are required for signaling downstream of FLS2 and EFR.

BAK1 belongs to a subclass of the subfamily II of LRR-RLKs, referred to as the SERK family based on sequence homology with the carrot (*Daucus carota*) LRR-RK SERK protein (Hecht et al., 2001). The SERK family contains five closely related members in *Arabidopsis*, with BAK1 corresponding to SERK3. The *Arabidopsis* SERK proteins are involved in diverse signaling pathways and are often functionally redundant (Albrecht et al., 2008). In addition to BAK1, SERK1 and BAK1-LIKE1/SERK4 (BKK1/SERK4) also interact with BRI1 as positive regulators of BR responses (Karlova et al., 2006; He et al., 2007; Albrecht et al., 2008; Jeong et al., 2010). Furthermore, SERK1 and SERK2 have redundant roles in male sporogenesis (Albrecht et al., 2005, 2008; Colcombet et al., 2005), and SERK1 has recently been shown to be involved in organ separation in flowers (Lewis et al., 2010). Importantly, BKK1 and BAK1 are both required to regulate cell death and senescence (He et al., 2007; Kemmerling et al., 2007; Jeong et al., 2010).

Here, we demonstrate that EFR forms a ligand-induced complex with BAK1 in vivo. In addition, we show the ligand-induced recruitment of additional SERKs in the EFR and FLS2 heterooligomeric complexes. Using a novel *bak1* allele that does not exhibit defects in BR and cell death responses (Schwessinger et al., 2011), we determined that BAK1 and BKK1 cooperate genetically to regulate multiple PRR-mediated signaling pathways. Furthermore, we demonstrate that BAK1 and BKK1 contribute to disease resistance against hemibiotrophic bacteria and an obligate biotrophic oomycete. Our work sheds light on the molecular events that immediately follow PAMP perception in plants and their contribution to innate immunity.

RESULTS

EFR and BAK1 Interact in a Specific Ligand-Induced Manner

To test if EFR heterodimerizes with BAK1 in *Arabidopsis*, we used transgenic plants expressing the functional EFR-enhanced green fluorescent protein–haemagglutinin (eGFP-HA) fusion protein under the control of the native *EFR* promoter in the null *efr-1* mutant background (*efr-1*/ProEFR:EFR-eGFP-HA; Nekrasov et al., 2009) in coimmunoprecipitation experiments. BAK1 was detected using recently developed anti-BAK1 peptide antibodies (Schulze et al., 2010). While no BAK1 could be detected above background level in the anti-GFP immunoprecipitate derived from the untreated transgenic EFR-eGFP-HA samples, a strong band was seen following 5 min of elicitation with elf18 (Figure 1A). These results suggest a ligand-induced complex formation between EFR and BAK1 in *Arabidopsis*.

Although specifically targeted against BAK1, the anti-BAK1 antibodies could potentially cross-react with BKK1 and SERK5 (Schulze et al., 2010). The specificity of the antibodies was further studied by immunoblotting total proteins extracted from wild-type Columbia-0 (Col-0) or null mutant *bak1-4* seedlings. A specific band of around the expected size of 75 kD was detected in the Col-0 extract that was only faintly detectable in the *bak1-4* total protein extract (see Supplemental Figure 1 online). However, if anti-BAK1 immunoprecipitation was performed on protein extracts from Col-0 and *bak1-4*, a band was clearly detectable in the Col-0 immunoprecipitate and a weaker band was found in the *bak1-4* immunoprecipitate (see Supplemental Figure 1 online). This suggests that the antibody likely cross-reacts weakly with BKK1 and potentially other SERKs in *Arabidopsis* seedling total extracts and immunoprecipitates.

To circumvent the problem of cross-reactivity and to test unambiguously if BAK1 can form a ligand-induced complex with EFR, we first designed epitope-tagged constructs for FLS2, EFR, or BAK1. These were then expressed transiently in leaves of the model plant *N. benthamiana* that allows *Agrobacterium*-mediated

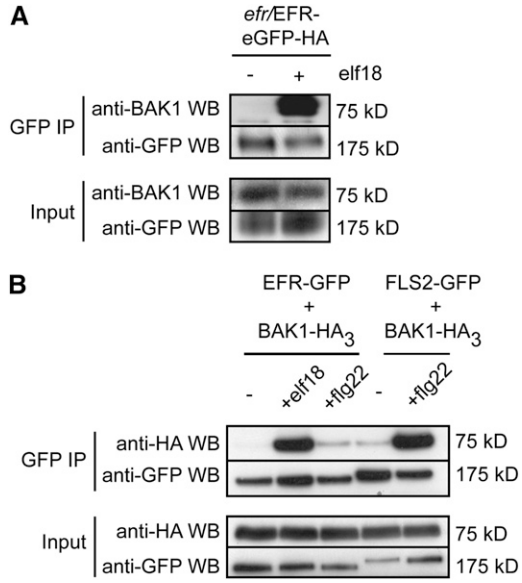

Figure 1. EFR and BAK1 Interact in a Specific Ligand-Induced Manner.

(A) Coimmunoprecipitation of EFR and BAK1. Transgenic *efr-1 Arabidopsis* seedlings expressing EFR-eGFP-HA under the native promoter were treated (+) or not (−) with 100 nM elf18 for 5 min. Total proteins (input) were subjected to immunoprecipitation with GFP Trap beads followed by immunoblot analysis with anti-BAK1 antibodies to detect BAK1 and anti-GFP antibodies to detect EFR-eGFP-HA.

(B) Coimmunoprecipitation of EFR or FLS2 and BAK1. *N. benthamiana* leaves expressing BAK1-HA_3 and EFR-GFP or FLS2-GFP were treated (+) or not (−) with 100 nM elf18 or flg22 for 5 min. Total proteins (input) were subjected to immunoprecipitation with GFP Trap beads followed by immunoblot analysis with anti-HA antibodies to detect BAK1-HA_3 and anti-GFP antibodies to detect EFR-GFP or FLS2-GFP.

Molecular mass is indicated in kilodaltons. These experiments were repeated three times with similar results.

transient coexpression of defined tagged proteins (Goodin et al., 2008). First, we verified that the previously reported flg22-induced FLS2-BAK1 interaction (Chinchilla et al., 2007; Heese et al., 2007; Schulze et al., 2010) could be recapitulated in *N. benthamiana*. After coexpression of GFP epitope–tagged FLS2 (FLS2-GFP) and HA_3 epitope–tagged BAK1 (BAK1-HA_3), we could induce an interaction between FLS2 and BAK1, detectable within 5 min of flg22 elicitation (Figure 1B). Consistent with the ligand dependency of the FLS2-BAK1 association (Chinchilla et al., 2007; Heese et al., 2007; Schulze et al., 2010) and the inactivity of the flg22 epitope derived from *Agrobacterium* (Felix et al., 1999; Bauer et al., 2001), the FLS2-BAK1 association was not detected in the absence of flg22 treatment above background level (Figure 1B). In addition, the well-characterized interaction between BRI1 and BAK1 (Li et al., 2002; Nam and Li, 2002) was similarly confirmed using GFP epitope–tagged BRI1 (BRI1-GFP) and BAK1-HA_3 and was enhanced by 3-h treatment with brassinolide (BL) (see Supplemental Figure 2 online). Thus, *N. benthamiana* is a useful system to study biologically relevant complex formation between different ligand binding RKs and BAK1.

While only a weak BAK1-HA_3 signal was observed in EFR-GFP immunoprecipitates in absence of its specific ligand (i.e., no ligand and flg22), a clear induction of oligomerization between EFR and BAK1 was observed 5 min after the addition of elf18 (Figure 1B; Schwessinger et al., 2011). Thus, similarly to the flagellin receptor, EFR needs to be activated by its ligand to heteromerize with BAK1.

Identification of Additional SERKs in the EFR Complex

To identify other proteins that form a ligand-induced complex with EFR, we analyzed the in vivo EFR complex composition by mass spectrometry. Anti-GFP immunoprecipitates were prepared from untreated and elf18-treated transgenic *efr-1*/EFR-eGFP-HA seedlings, as well as from untreated *efr-1* null mutant seedlings to reveal proteins that may bind nonspecifically to the GFP beads. Immunoprecipitated proteins were then separated by SDS-PAGE, gel slices were excised, and in-gel trypsin digestion was performed. Sequencing of tryptic peptides by liquid chromatography–tandem mass spectrometry (LC-MS/MS) identified 29 different peptides matching members of the SERK family. Importantly, these peptides were only present in the elf18-elicited transgenic sample (Table 1). Only peptides with a sufficiently high Mascot score (>20) were considered as a true indication of the presence of a particular SERK in the immunoprecipitates (Table 1). Given the high degree of identity among the SERK family (Hecht et al., 2001; Albrecht et al., 2008), it is difficult to unambiguously assign tryptic peptides to individual specific SERK proteins. After careful analysis of the identified peptides based on multiple alignments of the SERK proteins, two peptides unique to BAK1 were identified in all three biological replicates. For SERK2 and BKK1, only a single peptide specific for each could be identified for each in all three biological replicates (Table 1). No peptides specific to SERK1 or SERK5 were found. In addition to the SERKs detected in the EFR immunoprecipitates, peptides corresponding to 45 other proteins were detected.

These data support the ligand-induced recruitment of BAK1 into the EFR complex in *Arabidopsis* and suggest that BKK1 and SERK2 may also be recruited.

EFR and FLS2 Interact with Several SERKs in a Ligand-Induced Manner in *N. benthamiana*

To confirm the interaction between the different SERKs and EFR, we transiently coexpressed individual HA_3 epitope–tagged SERKs together with EFR-GFP in *N. benthamiana*. Equal amounts of EFR were pulled down using GFP Trap beads and probed for the presence of SERK-HA_3 using anti-HA immunoblotting. While some SERKs could sometimes be weakly detected in mock-treated samples, elf18 treatment significantly increased the amount of SERK1, SERK2, BAK1, and BKK1 detectable in the EFR immunoprecipitate (Figure 2A). However, no elf18-dependent increase in the amount of SERK5 present in the EFR immunoprecipitate could be observed (Figure 2A). These results suggest that EFR is capable of mounting a ligand-induced complex with SERK1, SERK2, BAK1, and BKK1 in *N. benthamiana* (Figure 2A).

Table 1. Identification of SERK Tryptic Peptides by HPLC-ESI-MS/MS Analysis of elf18-Treated EFR Immunoprecipitates

				SERK					
Peptide Sequence	*n* Peptide	Occurrence[a]	Best Mascot Score	1	2	3[b]	4[c]	5	Domain[d]
NmEGDALHSLR	1	1/3	37.8		+				N-ter
NAEGDALSALK	8	3/3	71.7			+			N-ter
ERPESQPPLDWPKR	2	1/3	51.3			+			N-ter
NAEGDALTQLK	2	1/3	68.5				+		N-ter
KPQDHFFDVPAEEDPEVHLGQLK	1	1/3	35.7			+	+		iJM
ELLVATDNFSNK	4	3/3	57.3				+		iJM
ELQVATDSFSNK	4	3/3	84.8		+				iJM
LmDYKDTHVTTAVR	11	2/3	74.7	+	+	+			Kinase
LRGFcmTPTER	1	1/3	23.6	+	+	+	+	+	Kinase
ERPPSQLPLAWSIR	1	1/3	55.3		+				Kinase
DGTLVAVKR	1	1/3	58.2	+	+	+			Kinase
LANDDDiMLLDWVK	1	1/3	60.9				+	+	Kinase
ERPEGNPALDWPK	1	1/3	70.7				+	+	Kinase
LmNYNDSHVTTAVR	2	1/3	51.5				+	+	Kinase
GRLADGTLVAVKR	2	2/3	54.3	+	+	+			Kinase
LaNDDDVmLLDWVK	2	1/3	71.0	+	+	+	+	+	Kinase
LLVYPYmANGSVAScLR	2	1/3	87.8	+	+	+	+	+	Kinase
MSEVVR	3	1/3	24.7	+	+	+	+	+	Kinase
LADGTLVAVKR	4	2/3	55.8	+	+	+			Kinase
LADGTLVAVK	4	1/3	96.6	+	+	+			Kinase
ERPESQPPLDWPK	7	2/3	61.5			+			Kinase
LADGnLVAVKR	7	2/3	83.9				+	+	Kinase
LADGnLVAVK	10	3/3	72.6				+	+	Kinase
GFcmTPTER	11	2/3	48.0	+	+	+	+	+	Kinase
GTIGHIAPEYLSTGK	13	2/3	84.9	+	+	+	+	+	Kinase
GTIGHIAPEYLSTGK	13	2/3	84.9	+	+	+	+	+	kinase
ELQVASDNFSNK	28	3/3	78.9			+			Kinase
KLESLVDAELEGK	1	1/3	90.1				+	+	C-ter
mLEGDGLAeR	8	2/3	57.2			+	+	+	C-ter

Peptides occurring in all three replicates are marked in bold. ESI, electrospray ionization.
[a]Reproducibility of specific tryptic peptides out of three independent biological replicates.
[b]BAK1.
[c]BKK1.
[d]N-ter, N-terminal region; iJM, intracellular juxta-membrane region; kinase, kinase domain; C-ter, C-terminal extension.

To determine whether the SERKs nonspecifically adhere to GFP Trap beads, HA-tagged SERK1, SERK2, BAK1, and BKK1 were independently coexpressed with eGFP in *N. benthamiana*. The eGFP protein was pulled down with GFP Trap beads, and the immunoprecipitates were probed with anti-HA antibodies to detect the SERKs. In each case, the SERK-HA signal was present in the input but not the GFP immunoprecipitates (see Supplemental Figure 3 online). As a positive control for the same experiment, BAK1-HA_3 was coexpressed with EFR-GFP in *N. benthamiana*. EFR-GFP could be detected in the immunoprecipitate, and BAK1-HA_3 was only detectable following elicitation with 100 nM elf18 (see Supplemental Figure 3 online). Thus, SERK-HA does not nonspecifically adhere to the GFP Trap beads. Overall, our results demonstrate that EFR and BAK1 form a ligand-induced complex in planta.

FLS2 is so far only known to heteromerize with BAK1 (Chinchilla et al., 2007; Heese et al., 2007). To test if FLS2 is also capable of interacting with additional SERKs, we transiently coexpressed individual HA_3 epitope–tagged SERKs together with FLS2-GFP in *N. benthamiana*. Equal amounts of FLS2-GFP-His protein could be immunoprecipitated using GFP Trap beads (Figure 2B). As observed with EFR, all SERKs were weakly detectable in FLS2 immunoprecipitates even in the absence of elicitation (Figure 2B). However, the association of FLS2 with SERK2 and BAK1, and to a lesser with SERK1 and BKK1, could be enhanced by 5 min of flg22 treatment (Figure 2B). Interestingly, EFR seemed to heteromerize equally strongly with SERK1, SERK2, BAK1, and BKK1 after elf18 treatment (Figure 2A), while a less marked flg22-dependent increase in the FLS2 association with SERK1, SERK2, and BKK1 could be detected (Figure 2B). Therefore, FLS2 showed a preferential heteromerization with BAK1 (Figure 2B) that correlates nicely with their respective genetic dependency on BAK1 (Chinchilla et al., 2007; Schwessinger et al., 2011). The weak heteromerization with SERK5 could not be enhanced by treatment with either ligand (Figures 2A and 2B).

These data suggest that FLS2 is also capable of interacting in a ligand-induced manner with SERK1, SERK2, BAK1, and BKK1 in *N. benthamiana* but that in this heterologous system, BAK1 is the

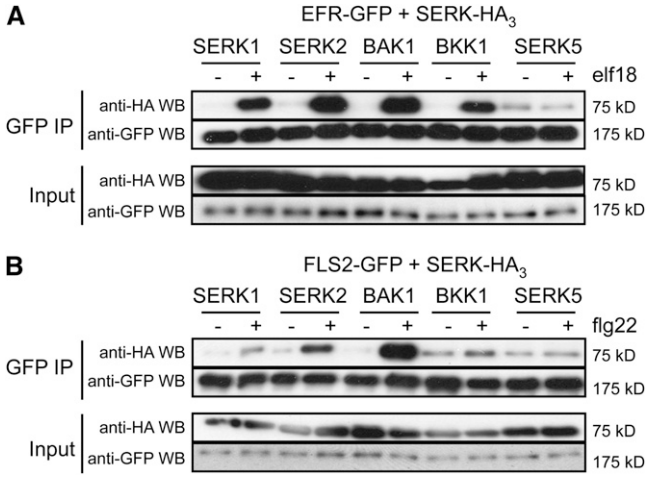

Figure 2. EFR and FLS2 Interact with Several SERK Proteins in a Ligand-Induced Manner in *N. benthamiana*.

(A) Coimmunoprecipitation of EFR and SERK proteins. *N. benthamiana* leaves expressing SERK-HA_3 constructs and EFR-GFP were treated (+) or not (−) with 100 nM elf18 for 5 min. Total proteins (input) were subjected to immunoprecipitation with GFP Trap beads followed by immunoblot analysis with anti-HA antibodies to detect SERK-HA_3 and anti-GFP antibodies to detect EFR-GFP.

(B) Coimmunoprecipitation of FLS2 and SERK proteins. *N. benthamiana* leaves expressing SERK-HA_3 constructs and FLS2-GFP were treated (+) or not (−) with 100 nM flg22 for 5 min. Total proteins (input) were subjected to immunoprecipitation with GFP Trap beads followed by immunoblot analysis with anti-HA antibodies to detect SERK-HA_3 and anti-GFP antibodies to detect FLS2-GFP.

Molecular mass is indicated in kilodaltons. These experiments were repeated three times with similar results.

preferred interactor of FLS2, while EFR shows less selectivity for a particular protein among these SERKs.

EFR and FLS2 Interact with Several SERKs in a Ligand-Induced Manner in *Arabidopsis*

In order to confirm the heteromerization of SERKs with EFR and FLS2 in *Arabidopsis*, we made use of recently available transgenic lines that express each of the SERKs tagged with a C-terminal Flag epitope (Gou et al., 2010). Due to very low expression of the SERK2-Flag transgenic line, we omitted SERK2-Flag from this study. To study EFR-SERK interactions, we created double transgenic lines by crossing the EFR-eGFP line with each individual SERK-Flag transgenic line. Equal amounts of SERK1, BAK1, BKK1, and SERK5 could be immunoprecipitated from each of the F1 lines using anti-Flag immunoaffinity resin (Figure 3A). In each case, the anti-Flag antibody detected a strong band at the correct size expected for the tagged SERKs, and this was absent from EFR-eGFP seedlings (Figure 3A). Anti-GFP antibodies detected EFR-eGFP (around 175 kD) in immunoprecipitates derived from elf18-treated SERK1, BAK1, and BKK1-expressing tissue (Figure 3A). This was not the case for SERK5-Flag, which was not associated with EFR-eGFP (Figure 3A), as previously observed in *N. benthamiana* (Figure 2A). Thus, we confirmed that in *Arabidopsis* EFR is also capable of ligand-induced interaction with SERK1, BAK1, and BKK1.

The transgenic SERK-Flag overexpressing lines were used to assess the ability of native *Arabidopsis* FLS2 to interact with diverse SERKs in vivo. Anti-FLAG immunoaffinity resin was used to immunoprecipitate equal amounts of SERK-Flag proteins before and after flg22 elicitation (Figure 3B). Anti-FLS2 antibodies were then used to detect endogenous FLS2 in the Flag immunoprecipitates. As a negative control, beads alone were incubated with protein extracts of an individual SERK-Flag line, and this did weakly cross-react with anti-FLS2 antibodies, constituting the background level (Figure 3B, lane 1). By contrast, a clear FLS2 signal was detected in SERK1, BAK1, and BKK1 anti-Flag immunoprecipitates derived from flg22-treated tissue (Figure 3B). This suggests a PAMP-induced association between FLS2 and SERK1, BAK1, and BKK1. Similar to EFR (Figure 3A)

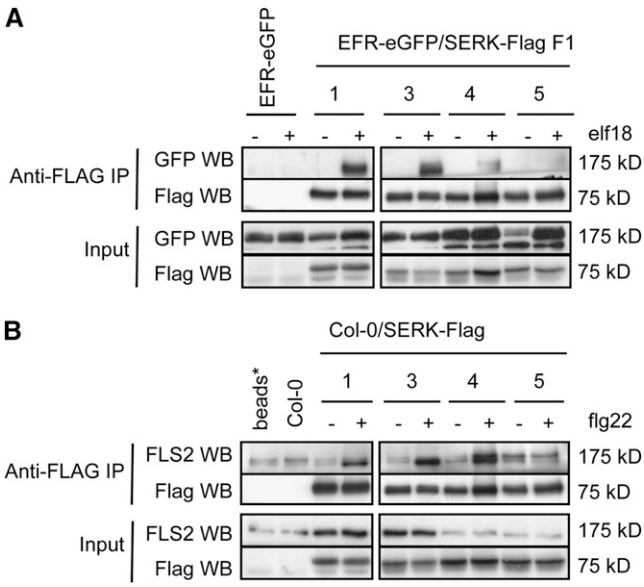

Figure 3. EFR and FLS2 Interact with Several SERK Proteins in a Ligand-Induced Manner in *Arabidopsis*.

(A) Coimmunoprecipitation of EFR-eGFP and SERK proteins. Transgenic *Arabidopsis* leaves expressing EFR-eGFP or EFR-eGFP and SERK-Flag were treated (+) or not (−) with 100 nM elf18 for 5 min. Total proteins (input) were subjected to immunoprecipitation with anti-FLAG immunoaffinity beads followed by immunoblot analysis with anti-Flag antibodies to detect SERK-Flag and anti-GFP antibodies to detect EFR-eGFP.

(B) Coimmunoprecipitation of FLS2 and SERK proteins. Transgenic *Arabidopsis* leaves expressing SERK-Flag were treated (+) or not (−) with 100 nM flg22 for 5 min. Total proteins (input) were subjected to immunoprecipitation with anti-FLAG immunoaffinity beads followed by immunoblot analysis with anti-Flag antibodies to detect SERK-Flag and anti-FLS2 antibodies to detect endogenous FLS2. Beads without antibodies (asterisk) were used as a control to illustrate that FLS2 does not nonspecifically adhere to immunoprecipitate beads (lane 1), and untransformed Col-0 *Arabidopsis* tissue was used as a control to illustrate specific detection of SERK-Flag proteins by anti-Flag antibodies (lane 2). Molecular mass of detected proteins indicated in kilodaltons.

and as observed in *N. benthamiana* (Figure 2B), no ligand-enhanced association was detected between FLS2 and SERK5 (Figure 3B). Notably, we tried to compare the properties of the heteromerization between EFR or FLS2 and BAK1 or BKK1 by fluorescence lifetime imaging microscopy–Förster resonance energy transfer microscopy in *Arabidopsis* protoplasts. However, the expression levels of EFR and BKK1 were too low to enable any meaningful measurements.

In conclusion, as in *N. benthamiana*, EFR and FLS2 are capable of forming a ligand-induced heterooligomer with at least SERK1, BAK1, and BKK1 in *Arabidopsis*.

BAK1 and BKK1 Are Required for EFR-, FLS2-, and PEPR1/2-Dependent Responses

Our biochemical analyses revealed that SERK1, SERK2, BAK1, and BKK1 can form a ligand-induced complex with EFR and FLS2 in vivo (Figures 1 to 3, Table 1). We then sought to test genetically the biological relevance of these SERKs for EFR- and FLS2-dependent signaling. In agreement with previous reports (Chinchilla et al., 2007; Heese et al., 2007), we showed that, with the exception of *bak1* mutants, individual *serk* null mutants were not significantly impaired in flg22 and elf18 responses as measured by the production of a ROS burst and seedling growth inhibition (see Supplemental Figure 4 online). The absence of observable phenotypes in single *serk* mutants could be due to functional redundancy between the related SERK proteins (Albrecht et al., 2008).

To test if SERK1 and SERK2 cooperate with BAK1 to regulate EFR- and FLS2-dependent signaling, we generated double mutants between the null mutant *bak1-4* (Chinchilla et al., 2007) and the null mutants *serk1-3* (Albrecht et al., 2008) and *serk2-2* (Albrecht et al., 2005) and tested their responsiveness to flg22 and elf18. The double mutants *serk1-3 bak1-4* and *serk2-2 bak1-4* were not further impaired than the *bak1-4* single mutants in the ROS burst triggered by flg22 and elf18 perception (Figure 4).

The seedling lethality of the *bak1-4 bkk1-1* double mutant due to uncontrolled cell death (He et al., 2007) prevented us from testing if BKK1 could play a role in elf18 and flg22 responses acting redundantly to BAK1. To overcome this technical limitation, we took advantage of the newly characterized semidominant *bak1-5* allele identified in a forward-genetic screen for *elf18-insensitive* (*elfin*) mutants (Nekrasov et al., 2009; Schwessinger et al., 2011). Interestingly, *bak1-5* is more severely impaired in elf18 and flg22 responses than the null mutant *bak1-4* (Schwessinger et al., 2011; Figure 5). Moreover, *bak1-5* is not impaired in BL responses and does not display uncontrolled cell death when combined with the null *bkk1-1* allele (Schwessinger et al., 2011). *bak1-5* still accumulates wild-type levels of BAK1-5 protein that harbors a single amino acid substitution (Cys to Tyr at position 408) in the kinase domain, which leads to reduced phosphorylation status (Schwessinger et al., 2011). However, the kinase activity of BAK1-5 is required to confer its phenotype, revealing a phosphorylation-dependent differential control of BR responses, cell death control, and PTI signaling (Schwessinger et al., 2011). This observation, together with the lack of direct correlation between the quantitative kinase output of BAK1 variants previously published and the responsiveness to flg22, reveals that perturbations of specific phosphosites on BAK1-5 or on associated FLS2 or EFR likely explain the *bak1-5* phenotype, potentially by affecting the recruitment or activation of downstream signaling components (Schwessinger et al., 2011).

As *bak1-5 bkk1-1* double mutant plants were fully viable and did not show any cell death–related phenotypes (Schwessinger et al., 2011), we used this double mutant to study the roles of BAK1 and BKK1 in EFR- and FLS2-dependent signaling. The *bak1-5* mutant showed strikingly reduced responses to both flg22 and elf18 in all assays conducted. Leaf discs from wild-type Col-0 *Arabidopsis* plants produced a ROS burst upon flg22 or elf18 addition, which was significantly reduced in *bak1-5* (Figure 5A). By contrast, *bkk1-1* exhibited a ROS burst comparable to wild-type leaf discs in response to both PAMPs (Figure 5A). Remarkably, leaf discs from *bak1-5 bkk1-1* plants displayed a negligible ROS burst in response to flg22 or elf18 (Figure 5A).

We then tested if the combination of the *bak1-5* and *bkk1-1* mutations would similarly impact other responses triggered by flg22 and elf18, which show a different temporal behavior. This was particularly relevant since the null mutant *bak1-4* was reported to be impaired in both early and late responses to flg22 but was not impaired in late responses (e.g., seedling growth inhibition) triggered by elf18 (Chinchilla et al., 2007). The *Arabidopsis* MAP kinases MPK3, MPK4, and MPK6 are activated within 5 min of flg22 and elf18 treatment (Zipfel et al., 2006; Figure 5B). MPK activation was reduced and delayed in *bak1-5* seedlings in comparison to the wild type and *bkk1-1* and was almost undetectable in *bak1-5 bkk1-1* seedlings in response to flg22 and elf18 over the time course assayed (Figure 5B).

The expression of >1000 genes is altered within 30 min of flg22 or elf18 treatment (Zipfel et al., 2004, 2006). The *bak1-4* mutation had only a minor effect on the expression of PAMP-induced marker genes *At1g51890* and *At2g17740* (He et al., 2006) after flg22 or elf18 treatment but reduced the expression of *At5g57220* after 3 h of flg22 treatment (Figure 5C). In agreement with the reduced impact of the *bak1-4* mutation on elf18 responses previously observed (Chinchilla et al., 2007; Figure 5A), the expression of these genes was not significantly altered in this

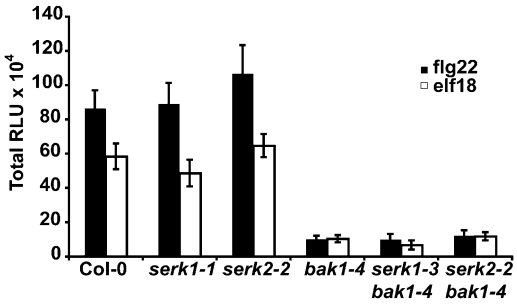

Figure 4. Mutations in *SERK1* or *SERK2* Do Not Enhance the *bak1-4* PTI Phenotype.

Total ROS production represented as RLUs of 4-week-old Col-0, *bak1-4*, *serk1-1*, *serk2-2*, *serk1-3 bak1-4*, and *bak1-4 serk2-2* after elicitation with 100 nM flg22 or elf18. Results are average ± SE (n = 8). These experiments were repeated four times with similar results.

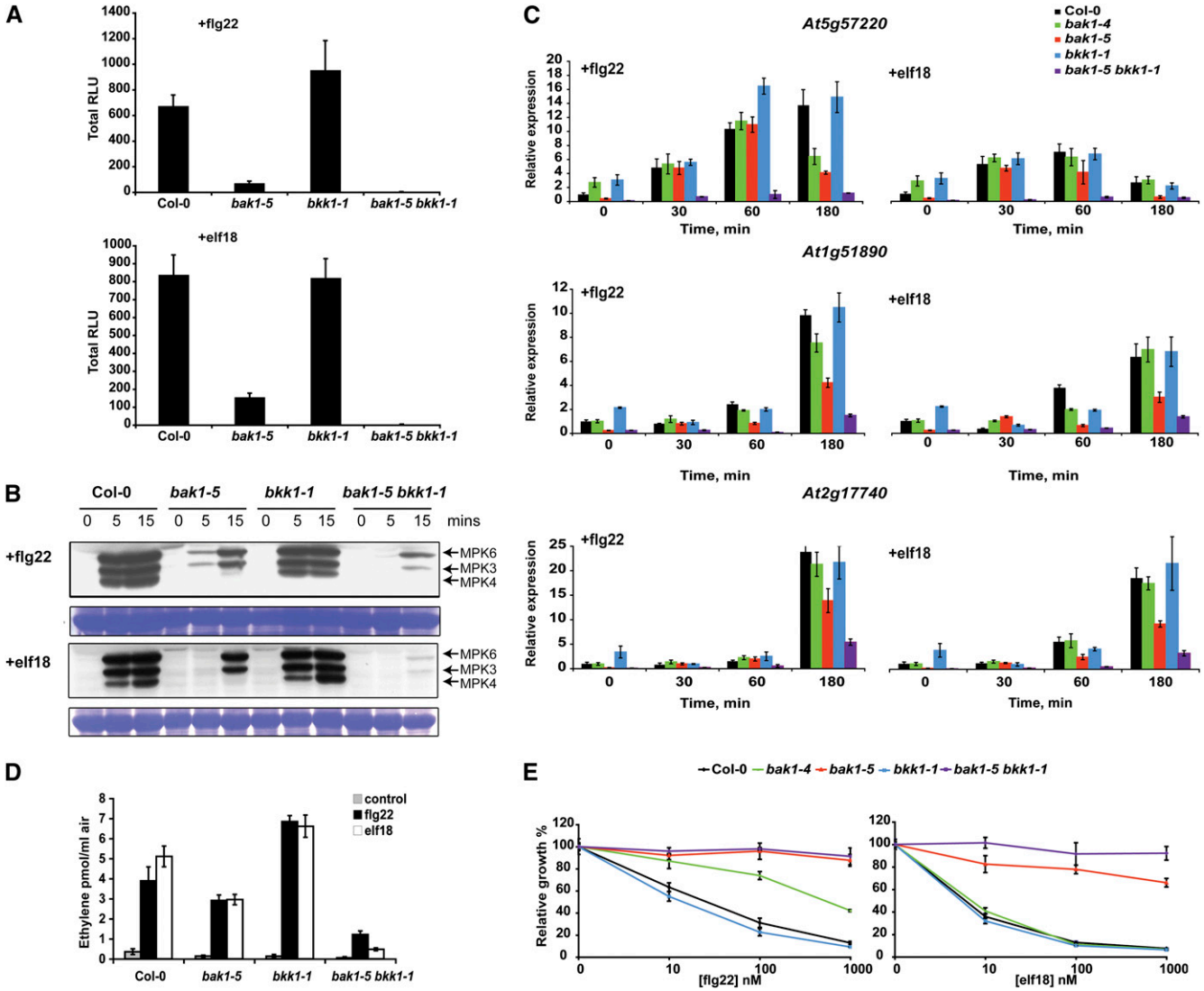

Figure 5. BAK1 and BKK1 Are Required for flg22 and elf18 Responses in *Arabidopsis*.

(A) Total ROS production over a period of 40 min represented as RLUs in Col-0, *bak1-5*, *bkk1-1*, and *bak1-5 bkk1-1* leaf discs after elicitation with 100 nM flg22 (top) or elf18 (bottom). Results are average ± SE (n = 8).

(B) Kinetics of MAPK activation after elicitation with 100 nM flg22 or elf18 in Col-0, *bak1-5*, *bkk1-1*, and *bak1-5 bkk1-1* seedlings as shown by immunoblot analysis using an anti-p44/42-ERK antibody. Immunoblot, top panel; Coomassie blue–stained membrane, bottom panel. The identity of individual MAP kinases as determined by size is indicated by arrows.

(C) Defense gene induction in response to 100 nM flg22 or elf18 of Col-0, *bak1-4*, *bak1-5*, *bkk1-1*, and *bak1-5 bkk1-1* seedlings. Gene expression of *At2g17740*, *At5g57220*, and *At1g51890* was measured by quantitative PCR analysis, normalized to *UBQ10* (reference gene) expression, and plotted relative to Col-0 at the 0 min expression level. Results are average ± SE (n = 3).

(D) Ethylene production in Col-0, *bak1-5*, *bkk1-1*, and *bak1-5 bkk1-1* leaves after mock (gray bars), 100 nM flg22 (black bars), or elf18 (white bars) treatments. Results are average ± SE (n = 6).

(E) Seedling growth inhibition triggered by flg22 or elf18 in Col-0, *bak1-4*, *bak1-5*, *bkk1-1*, and *bak1-5 bkk1-1* seedlings. Growth is represented relative to the untreated wild type. Results are average ± SE (n = 6).

All experiments were repeated at least three times with similar results.

mutant after elf18 treatment (Figure 5C). However, the induction of the three genes was reduced in response to either PAMP in *bak1-5* in comparison to the wild type, *bkk1-1*, or *bak1-4* (Figure 5C). The expression of these genes in *bkk1-1* was comparable to that in the wild type (Figure 5C). Strikingly, the expression of these genes was only minimally induced after flg22 or elf18 treatment in *bak1-5 bkk1-1* (Figure 5C).

An increase in ethylene biosynthesis can be measured in leaves within 2 h of treatment with flg22 or elf18 (Felix et al., 1999; Kunze et al., 2004). A clear flg22- or elf18-induced ethylene

production was measured in Col-0 and *bkk1-1* plants (Figure 5D). By contrast, this was significantly reduced in *bak1-5* leaves, and only marginal ethylene production could be measured in *bak1-5 bkk1-1* in response to flg22 or elf18 (Figure 5D). Interestingly, the ROS burst and ethylene production triggered by flg22 and elf18 was sometimes higher in *bkk1-1* leaves than in Col-0 leaves, which may be explained by the weak constitutive cell death and early senescence of this mutant (He et al., 2007; Jeong et al., 2010).

Seedling growth inhibition following days of treatment with flg22 or elf18 is a commonly used marker of PAMP response (Gómez-Gómez et al., 1999; Gómez-Gómez and Boller, 2000; Zipfel et al., 2006). As shown in Figure 5E, seedling growth inhibition in *bkk1-1* was comparable to that in the wild type, while *bak1-4* seedlings were only impaired in the growth inhibition triggered by flg22, as previously reported (Chinchilla et al., 2007). In comparison to *bak1-4*, *bak1-5* seedlings were further affected in the growth inhibition triggered by flg22 and were significantly affected in the growth inhibition triggered by elf18 (Figure 5E). Notably, the seedling growth inhibition triggered by elf18 was even further decreased in *bak1-5 bkk1-1* seedlings (Figure 5E). The combination of the *bak1-5* and *bkk1-1* mutations rendered these plants insensitive to long-term exposure to a high concentration (1 μM) of either PAMP (Figure 5E), a feature previously only associated with mutations affecting the receptors themselves (Gómez-Gómez and Boller, 2000; Zipfel et al., 2006; Li et al., 2009; Saijo et al., 2009). Notably, the combination of *bak1-5* with *serk1-1* or *serk2-1* did not further decrease the sensitivity to elf18 in the SGI assay, in contrast with *bak1-5 bkk1-1* (see Supplemental Figure 5 online), further demonstrating that mutations in *SERK1* or *SERK2* do not enhance the *bak1-5* phenotype.

In summary, we showed that loss of *BKK1* further decreased early and late responses of *bak1-5* to elf18 and flg22. Notably, the fact that the *bak1-5* phenotype could be further increased by combining the *bkk1-1* null mutation also demonstrated that the phenotype of *bak1-5* is not linked to a dominant-negative effect on BKK1.

We then tested if BAK1 and BKK1 are also required for PEPR1/2-dependent signaling. We found that the Pep1-induced ROS burst is attenuated in *bak1-4* and further decreased in *bak1-5* and *bak1-5 bkk1-1* (Figure 6A). When measuring ethylene production, which is a later response, *bak1-5* leaves produced less ethylene than the wild type in response to Pep1, and *bak1-5 bkk1-1* leaves were insensitive to Pep1 in this assay (Figure 6B). *bkk1-1* leaves were not affected in their responsiveness to Pep1 (Figure 6). This experiment revealed that loss of *BKK1* in a *bak1-5* background also leads to a strong reduction in responsiveness to the DAMP Pep1.

Overall, our results demonstrate that both BAK1 and BKK1 are required to regulate EFR-, FLS2-, and PEPR1/2-dependent responses.

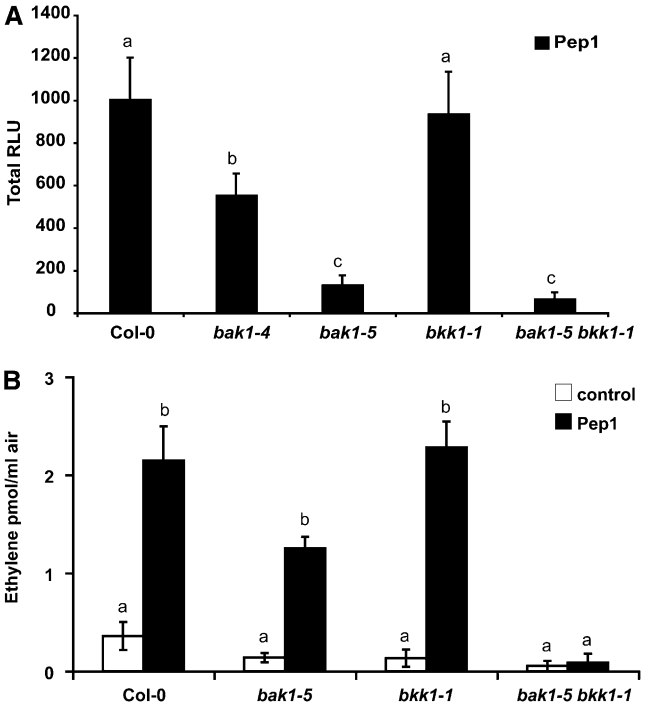

Figure 6. BAK1 and BKK1 Are Required for Pep1 Responses.

(A) Total ROS production over a period of 40 min represented as RLUs in Col-0, *bak1-4*, bak1-5, *bkk1-1*, and *bak1-5 bkk1-1* plants after elicitation with 100 nM Pep1. Results are average ± SE (n = 8).

(B) Ethylene production in Col-0, *bak1-5*, *bkk1-1*, and *bak1-5 bkk1-1* leaves after mock (white bars) or 100 nM Pep1 (black bars) treatments. Results are average± SE (n = 6).

All experiments were repeated at least three times with similar results. The letters above the graph denote statistically significant differences at $P < 0.0001$ (analysis of variance [ANOVA], Bonferroni post-test). All experiments were repeated at least three times with similar results.

BAK1 and BKK1 Are Required for Disease Resistance to Hemibiotrophic and Biotrophic Pathogens

Next, we assessed whether BAK1 and BKK1 contribute to disease resistance. We first infected plants with the highly virulent hemibiotrophic bacterium *Pto* DC3000. As reported previously (Zipfel et al., 2004; Nekrasov et al., 2009), *efr-1 fls2* plants are hypersusceptible to this strain upon spray inoculation (Figure 7A). The *bak1-4* and *bkk1-1* mutants, however, exhibited wild-type susceptibility levels (Figure 7A). By contrast, leaves of *bak1-5* and *bak1-5 bkk1-1* allowed greater growth of *Pto* DC3000 compared to *efr-1 fls2* (Figure 7A; Schwessinger et al., 2011).

PTI defects can be detected more sensitively with weakly virulent bacterial strains that lack effector molecules, such as AvrPto and AvrPtoB, or the phytotoxin coronatine, which is involved in PTI suppression (Melotto et al., 2006; Göhre et al., 2008; Shan et al., 2008; Xiang et al., 2008; Nekrasov et al., 2009). As shown in Figure 7B, *efr-1 fls2* mutants were more susceptible than the wild type to spray inoculation with *Pto* DC3000 Δ*AvrPto*/Δ*AvrPtoB*, while *bak1-4* plants were not. Interestingly, *bak1-5* and *bak1-5 bkk1-1* plants were hypersusceptible to *Pto* DC3000 Δ*AvrPto*/Δ*AvrPtoB*, whereas *bkk1-1* plants exhibited wild-type bacterial susceptibility (Figure 7B). Indeed, *Pto* DC3000 Δ*AvrPto*/Δ*AvrPtoB* grew to similar levels in *bak1-5 bkk1-1* as did the isogenic wild-type *Pto* DC3000 in Col-0 plants, showing that the these mutations almost completely restored the virulence defect

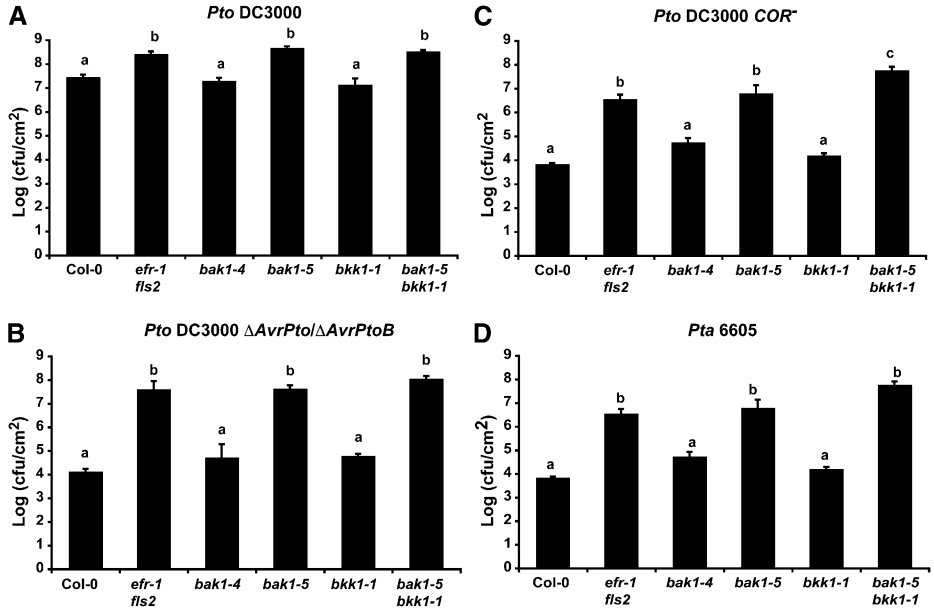

Figure 7. BAK1 and BKK1 Are Required for Resistance to Adapted and Nonadapted *Pseudomonas*.

(A) Four-week-old plants (Col-0, *efr-1 fls2*, *bak1-4*, *bak1-5*, *bkk1-1*, and *bak1-5 bkk1-1*) were spray inoculated with *Pto* DC3000 (OD_{600} = 0.02).
(B) Four-week-old plants (Col-0, *efr1 fls2*, *bak1-4*, *bak1-5*, *bkk1-1*, and *bak1-5 bkk1-1*) were spray inoculated with *Pto* DC3000 *ΔAvrPto/ΔAvrPto* (OD_{600} = 0.2).
(C) Four-week-old plants (Col-0, *efr1 fls2*, *bak1-4*, *bak1-5*, *bkk1-1*, and *bak1-5 bkk1-1*) were spray inoculated with *Pto* DC3000 COR^- (OD_{600} = 0.2)
(D) Four-week-old plants (Col-0, *efr1 fls2*, *bak1-4*, *bak1-5*, *bkk1-1*, and *bak1-5 bkk1-1*) were syringe inoculated with *P. syringae* pv *tabaci* 6605 (OD_{600} = 0.002) Bacterial counts were performed at 3 d after inoculation. Results are average ± SE (n = 4).
The letters above the graph denote statistically significant difference at $P < 0.0001$ (ANOVA, Bonferroni post-test). All experiments were repeated at least three times with similar results. cfu, colony-forming units.

associated with the loss of AvrPto and AvrPtoB. A similar pattern was observed when infecting with the *Pto* DC3000 COR^- strain. This strain already grew to higher levels in *efr-1 fls2* than in Col-0, *bak1-4*, or *bkk1-1* (Figure 7C). *bak1-5* plants were similarly hypersusceptible as *efr-1 fls2*, while this strain reproducibly colonized *bak1-5 bkk1-1* leaves to a greater extent than the *bak1-5* mutant or *efr-1 fls2* (Figure 7C), again reaching levels comparable to those observed with isogenic wild-type *Pto* DC3000 in Col-0 leaves (Figure 7A). Thus, BAK1 is a major contributor to the basal resistance to *Pto* DC3000, while BKK1 plays a less significant role that is only apparent in infections with the weakly virulent strain *Pto* DC3000 COR^-.

We then tested if BAK1 and BKK1 play a role in the nonhost resistance against the nonadapted bacterium *P. syringae* pv *tabaci* 6605 (*Pta* 6605), which partially depends on FLS2 (Li et al., 2005; Figure 7D). Growth in *bak1-4* and *bkk1-1* reached similar low levels as in wild-type Col-0, while the *efr-1 fls2* mutant was significantly more susceptible, supporting up to 2 logs more bacterial growth than Col-0 (Figure 7D). The *bak1-5* and *bak1-5 bkk1-1* mutants were as susceptible to this nonadapted strain as the *efr-1 fls2* double mutant (Figure 7D), suggesting that nonhost resistance to *Pta* 6605 is compromised in the absence of functional BAK1.

Next, we assessed the role of BAK1 and BKK1 in resistance to the obligate biotrophic oomycete pathogen *Hyaloperonospora arabidopsidis* (*Hpa*). We first performed infections with the virulent isolate Emco5, which develops abundant sporangiophores and undergoes spore formation within 7 d of inoculation on *Arabidopsis* Col-0 seedlings (McDowell et al., 2005; Figure 8A). In comparison to Col-0, we observed decreased sporulation in *bak1-4* seedlings (Figure 8A), probably due to their increased cell death phenotype upon infection (Kemmerling et al., 2007). By contrast, no decrease in the number of spores could be observed in *bak1-5*, *bkk1-1*, or *bak1-5 bkk1-1* (Figure 8A). The absence of noticeable phenotype of *bak1-5 bkk1-1* seedlings could be due to the already high susceptibility of Col-0 to *Hpa* Emco5 that may mask a contribution of PTI. We thus decided to perform infections with *Hpa* isolates that are only weakly virulent on Col-0 seedlings. Sporulation of the *Hpa* isolate Cala2 on Col-0 seedlings is rare due to RESISTANCE TO P. PARASITICA2 (RPP2)-based resistance (Sinapidou et al., 2004). Indeed, we observed only occasional conidiophore formation on Col-0 seedlings inoculated with *Hpa* Cala2 that never resulted in sporulation (Figure 8B). By contrast, *bak1-5* and *bkk1-1* seedlings appeared reproducibly more susceptible to this isolate, but only *bak1-5 bkk1-1* seedlings consistently showed statistically significant enhanced susceptibility to *Hpa* Cala2 (Figure 8B). Additionally, infection with another weakly virulent isolate, *Hpa* Emoy2, where resistance in Col-0 is provided by RPP4 (Holub et al., 1994), revealed a similar pattern. This was in stark contrast with *bak1-5*

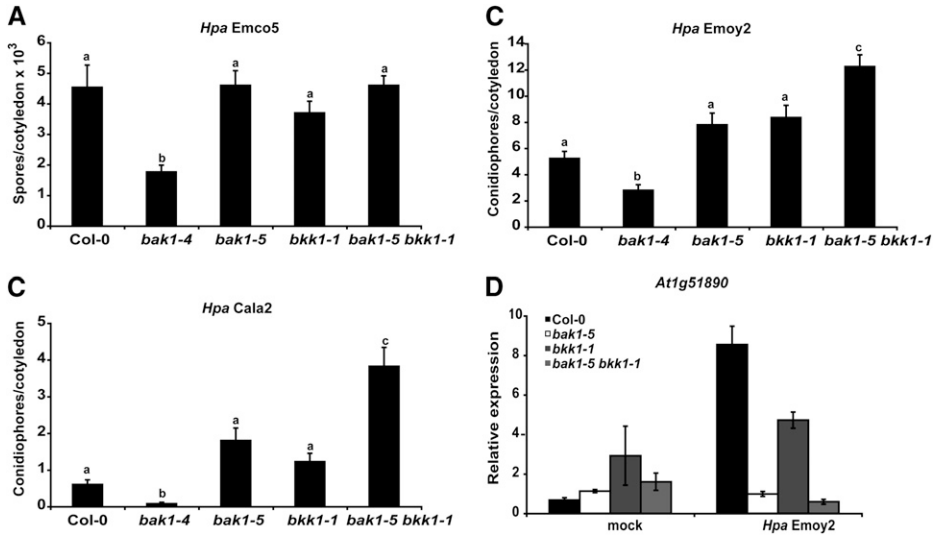

Figure 8. BAK1 and BKK1 Are Required for Resistance to the Obligate Biotrophic Oomycete Pathogen *H. arabidopsidis*.

(A) Infection of Col-0, *bak1-4*, *bak1-5*, *bkk1-1*, and *bak1-5 bkk1-1* seedlings with *H. arabidopsidis* Emco5. Spores were counted at 7 d after inoculation. Results are average ± SE (n = 12).

(B) Infection of Col-0, *bak1-4*, *bak1-5*, *bkk1-1*, and *bak1-5 bkk1-1* seedlings with *H. arabidopsidis* Cala2. Conidiophores were counted at 7 d after inoculation. Results are average ± SE (n = 40).

(C) Infection of Col-0, *bak1-4*, *bak1-5*, *bkk1-1*, and *bak1-5 bkk1-1* seedlings with *H. arabidopsidis* Emoy2. Conidiophores were counted at 7 d after inoculation. Results are average ± SE (n = 40). The letters above the graphs in **(A)** to **(C)** denote statistically significant difference at $P < 0.0001$ (ANOVA, Bonferroni post-test).

(D) Defense gene induction of Col-0, *bak1-5*, *bkk1-1*, and *bak1-5 bkk1-1* seedlings in response to a 3-h treatment with crude extracts of uninoculated (mock) or *Hpa* Emoy2-infected leaves. Gene expression of *At1g51890* was measured by quantitative PCR analysis, normalized to *UBQ10* (reference gene) expression, and plotted relative to the expression level in Col-0 at the initial time point. Results are average ± SE (n = 3).

All experiments were repeated at least three times with similar results.

bkk1-1 seedlings, where statistically significantly increased number of conidiophores could be counted (Figure 8C). As observed with the highly virulent isolate Emco5, *bak1-4* seedlings were less susceptible to the *Hpa* isolates Cala2 and Emoy2 (Figures 8B and 8C), as expected due to their deregulated cell death upon infection.

To determine whether the enhanced susceptibility of *bak1-5 bkk1-1*, and to a lesser extent *bak1-5*, seedlings to *Hpa* isolates observed in Figures 8A to 8C is due to compromised PTI induced by potential *Hpa* PAMPs, we analyzed the expression of the PTI marker gene *At1g51890* in Col-0, *bak1-5*, *bkk1-1*, and *bak1-5 bkk1-1* sterile seedlings treated with boiled extracts of mock- or *Hpa*-treated leaf tissue (Figure 8D). Treatment with the extract from *Hpa*-infected leaves led to the accumulation of *At1g51890* in Col-0, while treatment with the extract from mock-treated leaves did not (Figure 8D). By contrast, *bak1-5* and *bak1-5 bkk1-1* seedlings were almost insensitive to the extract from *Hpa*-infected leaves (Figure 8D). These results suggest that one or several *Hpa* PAMP(s) or DAMP(s) released during the infection may be detected in *Arabidopsis* and induce BAK1- and BKK1-dependent responses. Thus, the increased susceptibility of *bak1-5* and *bak1-5 bkk1-1* plants to *Hpa* is most likely due to defects in PTI. These results revealed a role for BAK1 and BKK1 in resistance to the obligate biotrophic oomycete *Hpa*.

DISCUSSION

The Regulatory LRR-RLK BAK1 Interacts in Planta with Several Ligand Binding LRR-RKs, Including EFR

Over the past few years, it has become evident that BAK1 is a versatile protein with roles in diverse signaling processes (Chinchilla et al., 2009). BAK1 was initially identified as an interactor of the LRR-RK BRI1 and a positive regulator of BR responses (Nam and Li, 2002; Li et al., 2002; Wang et al., 2005). However, it was recently shown that BAK1 also plays a BR-independent role as a positive regulator of PTI. BAK1 forms a rapid ligand-dependent complex with the PRR FLS2 and is required for full responsiveness to flg22 (Chinchilla et al., 2007; Heese et al., 2007; Schulze et al., 2010). In addition, *bak1* loss of function in *Arabidopsis* or *N. benthamiana* results in reduced responsiveness to several other PAMPs and DAMPs, including elf18, csp22, INF1, PGN, LPS, and AtPep1 (Chinchilla et al., 2007; Heese et al., 2007; Shan et al., 2008; Krol et al., 2010).

Furthermore, BAK1 and its closest paralog BKK1 are required for the regulation of light- and pathogen-induced cell death (He et al., 2007, 2008; Kemmerling et al., 2007; Jeong et al., 2010). In addition, the LRR-RLK BAK1-INTERACTING RECEPTOR KINASE1 (BIR1) interacts with BAK1 in vivo and is also necessary to regulate cell death (Gao et al., 2009). However, it is unclear

if the role of BAK1 and BKK1 in cell death regulation is linked to their interaction with any ligand binding RKs that perceive a hypothetical endogenous survival signal or if the integrity and/or activity of the BAK1/BIR1/BKK1-containing complex(es) is guarded by hypothetical R protein(s) (He et al., 2007; Kemmerling et al., 2007; Wang et al., 2008; Gao et al., 2009).

Despite these numerous examples of the genetic requirement of BAK1 in different pathways, the in vivo heteromerization of BAK1 with ligand binding RKs was so far only demonstrated for BRI1 and FLS2. Several results suggested that BAK1 may also form a ligand-induced complex with the PRR EFR. First, the null mutant *bak1-4* was affected in elf18-triggered early responses (Chinchilla et al., 2007; Shan et al., 2008). Second, elf18 treatment induced the phosphorylation of a band that coimmunoprecipitated with BAK1; this band has a similar size as the glycosylated form of EFR (Schulze et al., 2010). Together with another recent study from our laboratory (Schwessinger et al., 2011) using coimmunoprecipitation experiments in *Arabidopsis* and *N. benthamiana*, we demonstrated that EFR and BAK1 form a ligand-induced complex in vivo. This interaction occurred rapidly (<5 min) and was specific to elf18 treatment, similarly to the property of the FLS2-BAK1 association triggered by flg22. This provides only the third example of ligand-induced heteromerization between BAK1 and a ligand binding RK.

The PRRs EFR and FLS2 Form a Ligand-Induced Complex (es) with Multiple SERKs

Null *bak1* mutants are only partially insensitive to flg22 or elf18 (Chinchilla et al., 2007; Heese et al., 2007; Shan et al., 2008), suggesting that BAK1 is not the only rate-limiting component and that additional regulatory proteins are part of the FLS2 and EFR receptor complexes. Since BAK1/SERK3 is part of the multigenic SERK family, which contains five members, it is possible that additional SERKs associate with FLS2 and/or EFR in vivo. BRI1, for example, forms a ligand-induced complex with BAK1, but also with SERK1 and BKK1 (Li et al., 2002; Nam and Li, 2002; Karlova et al., 2006; He et al., 2007). Consistently, LC-MS/MS analysis of *Arabidopsis* anti-GFP immunoprecipitates from elf18-treated transgenic EFR-eGFP-HA seedlings identified specific peptides for SERK2, BAK1, and BKK1, suggesting that these three SERK proteins form ligand-induced complex(es) with EFR in vivo. Notably, 19 additional peptides matched multiple SERKs (Table 1), and seven peptides matched the highly similar BKK1 and SERK5. The presence of peptides that match several or all SERKs did not allow us to completely exclude the possibility that SERK1 and SERK5 may also be present in the EFR complex. Similarly, specific peptides corresponding to BAK1, SERK1, and SERK2 were also previously identified in HPLC-MS/MS analysis of the FLS2 immunocomplex in *Arabidopsis* (Heese et al., 2007). Accordingly, independent transient overexpression of epitope-tagged SERK and EFR proteins in *N. benthamiana* suggested that EFR is capable of mounting an elf18-induced heteromerization with SERK1, SERK2, BAK1, and BKK1. In parallel, the heteromerization between epitope-tagged SERKs and FLS2 was also tested. FLS2 was also capable of forming a ligand-induced complex with SERK1, SERK2, BAK1, and BKK1. However, the amount of SERK1 and BKK1 coimmunoprecipitated with FLS2 was very low. With both FLS2 and EFR, no ligand-induced association could be detected with SERK5. Our results thus suggest that FLS2 preferentially interacts with BAK1, and potentially SERK2, while EFR strongly interacts with SERK1, SERK2, BAK1, and BKK1. These results are in agreement with the fact that *bak1* null mutants are more strongly affected in flg22 than elf18 responses (Chinchilla et al., 2007).

In *Arabidopsis*, EFR and FLS2 interacted with SERK1, BAK1, and BKK1 in a ligand-induced manner. In this system, each SERK was overexpressed, while native levels of EFR or FLS2 were used. In contrast with the results observed in *N. benthamiana*, where all proteins were overexpressed, no preferential interaction was detected between FLS2 and BAK1 over FLS2 and other SERKs. This may due to a massive imbalance between protein levels of the overexpressed SERK proteins versus the endogenous ligand binding receptors, precluding a clear assessment of interaction affinities. Nonetheless, these results confirm that EFR and FLS2 are capable of interacting with at least SERK1, BAK1, and BKK1 in *Arabidopsis* in a ligand-induced manner. Furthermore, SERK5 did not interact with either FLS2 or EFR in this system, confirming the results from *N. benthamiana*.

The Regulatory LRR-RLKs BAK1 and BKK1 Are Important Regulators of FLS2-, EFR-, and PEPR1/2-Mediated Signaling

Having shown that several SERKs can form a ligand-induced complex with FLS2 and EFR, it was important to genetically test the importance of these SERKs for flg22 and elf18 responses. Transcripts of *SERK1*, *SERK2*, *BAK1*, and *BKK1* are upregulated in response to PAMP or pathogen treatments (Postel et al., 2010), supporting a potential role for these SERKs in innate immunity. As previously reported (Chinchilla et al., 2007; Heese et al., 2007), we found that apart from *bak1*, other single null *serk* mutants were not affected in flg22 and elf18 responses, as measured by the production of ROS (early response) and seedling growth inhibition (late response). These results did not disprove that other SERKs could play a role and could be explained by functional redundancy among different SERKs (Albrecht et al., 2008), in particular BAK1 in this case. Phenotypic analysis of double mutants between null alleles of *bak1* and *serk1* or *serk2* suggest that SERK1 and SERK2 do not play a role in FLS2- or EFR-triggered signaling, at least in the bioassays used in this study. The lack of phenotype associated with *serk1* and *serk2* single mutations, alone or in combination with *bak1-4* or *bak1-5*, may be due to functional redundancy between SERK1 and SERK2, as previously reported in the case of male sporogenesis (Albrecht et al., 2005, 2008; Colcombet et al., 2005). Alternatively, SERK1 and SERK2 may be predominantly expressed in cell types that are not necessarily relevant for the bioassays used in this study, which mostly relied on leaf tissues.

Testing the role of BKK1 in the absence of BAK1 is normally hindered by the fact that the double *bak1 bkk1* mutants show constitutive activation of cell death (He et al., 2007). To circumvent this problem, we took advantage of a new *bak1* allele, *bak1-5*, that is not impaired in BR signaling and that does not confer deregulated cell death when combined with *bkk1* mutations (Schwessinger et al., 2011). Importantly, early and late responses

to flg22 and elf18 were dramatically reduced in the double mutant *bak1-5 bkk1-1*. Interestingly, responses to the DAMP Pep1 were also severely impaired in *bak1-5 bkk1-1*, consistent with the BAK1 dependence of Pep1-triggered responses (Krol et al., 2010; Postel et al., 2010).

The fact that *bak1-5* was more impaired in flg22 and elf18 responses than *bak1-4* could suggest that this mutation has a dominant-negative effect on SERK1, SERK2, and/or BKK1. However the double mutants *bak1-4 serk1-3* and *bak1-4 serk2-2* were not less sensitive to flg22 and elf18 than *bak1-4*, indicating that SERK1 and SERK2 do not play a nonredundant role in FLS2 and EFR signaling pathways. In addition, the *bkk1-1* mutation further enhanced the *bak1-5* phenotype, suggesting that the BAK1-5 protein does not impair, at least completely, BKK1 function per se.

Our results thus reveal that BKK1 plays a major regulatory role in the FLS2-, EFR-, and PEPR1/2-dependent signaling pathways in addition to BAK1. The importance of both BAK1 and BKK1 in these pathways may reflect the existence of heterooligomeric complexes between these PRRs, BAK1 and BKK1. However, the exact composition and stoichiometry of these complexes remain to be determined.

BAK1 and BKK1 Are Required for Immunity to Hemibiotrophic and Obligate Biotrophic Pathogens

The role of BAK1 and, to a larger extent, BKK1, in plant disease resistance is unclear. An unambiguous analysis of the role of BAK1 and BKK1 in *Arabidopsis* disease resistance is hindered by the constitutive and pathogen-induced cell death phenotype of *bak1* and *bkk1* single and double null mutants (He et al., 2007; Kemmerling et al., 2007; Jeong et al., 2010). Accordingly, *bak1-4* plants exhibited pronounced chlorotic lesions upon infection with the hemibiotrophic bacterium *Pto* DC3000 but were not more susceptible to this bacterium (Kemmerling et al., 2007). Concomitantly, the same mutant plants were more resistant to the obligate biotrophic oomycete *Hpa* but more susceptible to the necrotrophic fungi *Botrytis cinerea* and *Alternaria brassicicola* (Kemmerling et al., 2007).

Intriguingly, silencing of *SERK3/BAK1* in *N. benthamiana* resulted in a clear hypersusceptibility to the adapted oomycete *Phytophthora infestans*, the adapted bacterium *Pta* 11528, and the nonadapted bacterium *Pto* DC3000 (Heese et al., 2007; Chaparro-Garcia et al., 2011). In addition, *SERK3/BAK1* silencing in tomato led to loss of *Verticillium* resistance mediated by the LRR receptor-like protein Ve1 (Fradin et al., 2009). Several nonmutually exclusive hypotheses could explain the strong impact of *SERK3/BAK1* silencing on disease resistance in *N. benthamiana* and tomato without an apparent impact on cell death regulation as observed in *Arabidopsis*. First, the silenced gene may not correspond to the true functional ortholog of *Arabidopsis BAK1*. Second, the silencing fragment may affect the expression of additional *SERK* paralogs whose function and/or identities are currently unknown. Finally, a hypothetical R protein(s) guarding the BAK1-BKK1 complex integrity and/or activity may not be present in *N. benthamiana* and tomato. Thus, silencing of *SERK3/BAK1* in these plants does not result in observable cell death phenotypes.

Another issue in interpreting the role of BAK1 in disease resistance is that the impact of BR signaling in defense is still unclear (Divi and Krishna, 2009). Consequently, conclusions on disease susceptibility of *bak1* mutants always need to be carefully weighed as these lines exhibit defects in hormone signaling, innate immunity, and cell death.

We took advantage of the *bak1-5* and *bak1-5 bkk1-1* lines to address the potential role of BAK1 and BKK1 in PTI against hemibiotrophic bacteria and the obligate biotrophic oomycete *Hpa*. Our results provide evidence of a role for BAK1 and BKK1 in *Arabidopsis* basal and nonhost resistances to *P. syringae* strains. *Bak1-5* mutants were more susceptible to several strains of *Pto* DC3000 and to the nonadapted strain *Pta* 6605. More importantly, our results also revealed that BAK1 and BKK1 are involved in resistance to *Hpa*. Surprisingly, the isolates Cala2 and Emoy2 that are normally resisted by the R proteins RPP2 and RPP4, respectively (Sinapidou et al., 2004; Holub, 2008), grew to a certain extent in *bak1-5 bkk1-1* seedlings. This may suggest that BAK1 and BKK1 are involved in effector-triggered immunity. Alternatively, this could reflect enhanced growth permitted by the lack of PTI in these lines. It is possible that this phenotype is observable only in interactions where the pathogen is weakly virulent. This latter hypothesis is actually supported by the reduced responsiveness of *bak1-5 bkk1-1* plants to a crude boiled extract from *Hpa*-infected *Arabidopsis* leaves (Figure 8D). Therefore, we speculate that BAK1 and BKK1 might also interact with an as yet unidentified PRR(s) for oomycete PAMP(s). Similarly, *bak1-5 bkk1-1* leaves were more susceptible than *efr-1 fls2* to the hypovirulent bacterial strain *Pto* DC3000 *COR*$^{-}$, indicating that at least another PAMP, other than EF-Tu or flagellin, derived from this bacterium is recognized by a BAK1/BKK1-dependent PRR. The identification of these novel PAMPs and corresponding receptors represents an interesting challenge for the future.

METHODS

Plant Materials and Growth Conditions

Arabidopsis thaliana ecotype Col-0 was the background for all mutants and transgenic lines used in this study. The *Arabidopsis* plants used in this study were grown as four plants per pot (9 × 9 cm) at 20 to 21°C with a 10-h photoperiod or on plates containing Murashige and Skoog (MS) salt medium (Duchefa), 1% Suc, and 1% agar with a 16-h photoperiod. The *efr*/EFRp-EFR-eGFP-HA lines were described by Nekrasov et al. (2009). The mutants *bak1-4* (Salk_116202), *bkk1-1* (Salk_057955), *serk1-1* (Salk_044330), *serk1-3* (GK_448E10), *serk2-2* (SAIL_119-G03), and *serk5-1* (SALK_147275) have been described elsewhere (Heese et al., 2007; Albrecht et al., 2008). The *bak1-5* mutant was identified in an ethyl methanesulfonate screen for *elfin* mutants (Nekrasov et al., 2009; Schwessinger et al., 2011).

The Pro35S:SERK-Flag lines were described by Gou et al. (2010).

Oxidative Burst Assay

Plants were grown for 4 weeks before sampling 20-mm leaf discs from eight plants per line. Leaf discs were placed in white 96-well plates (Greiner Bio-one) with water overnight. The following day, the water was removed and replaced with a solution of 17 mg/mL (w/v) luminol (Sigma-Aldrich) and 10 mg/mL (w/v) horseradish peroxidase (Sigma-Aldrich) containing 100 nM flg22, elf18, or AtPep1. The plates were analyzed for a

period of 40 min using a Varioskan Flash (Thermo Scientific) multiplate reader or a Photek camera. The amount of relative light units might differ depending on the light capturing apparatus used. In some graphs, the total amount of relative light units (RLUs) measured over a period of 40 min was plotted.

MAP Kinase Assay

Proteins were extracted from 14-d-old seedlings treated with water, 100 nM elf18, or 100 nM flg22 for 0, 5, or 15 min. Seedlings were ground in liquid nitrogen, and protein extraction buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 15 mM EGTA, 10 mM $MgCl_2$, 1 mM NaF, 1 mM $Na_2MoO_4 \cdot 2H_2O$, 0.5 mm $NaVO_3$, 30 mM β-glycerophosphate, 0.1% IGEPAL CA 630, 100 nM calyculin A [CST], 0.5 mM PMSF, and 1% protease inhibitor cocktail [Sigma-Aldrich; P9599]) was added. Homogenates were clarified by centrifugation and filtered through Miracloth. Proteins (40 μg) were diluted with loading buffer and subjected to SDS-PAGE and electroblotting. Immunoblots were blocked by incubation in 5% (w/v) BSA in TBS-Tween (0.1%) for 1 h. Activated MAP kinases were detected by overnight incubation with anti-p42/44 MAPK primary antibodies (1:1000; Cell Signaling Technology), followed by incubation with anti-rabbit-HRP secondary antibodies (Sigma-Aldrich) for 1 h.

Quantitative RT-PCR

Fourteen-day-old seedlings grown in MS medium were treated in triplicate with water or 100 nM elf18 or flg22 for 0, 30, 60, or 180 min before pooling them for harvesting. Total RNA was extracted from seedlings using the RNeasy plant mini kit (Qiagen). RNA samples were treated with DNase Turbo DNA-free (Ambion), quantified with a Nanodrop spectrophotometer (Thermo Scientific), and reverse transcribed into cDNA with SuperScript III reverse transcriptase (Invitrogen). SybrGreen master mix (Sigma-Aldrich) was used for quantitative PCR reactions. The *UBQ10* (*At4g05320*) gene was used for normalization. Gene expression of *At2g17740* (DC1 domain-containing protein), *At5g57220* (CYP81F2), and *At1g51890* (LRR-RK) was measured by quantitative PCR analysis, normalized to *UBQ10* (reference gene) expression, and plotted relative to Col-0 expression level at 0 min.

Ethylene Measurement

Plants were grown for 6 weeks before sampling 2-mm leaf strips from four plants per genotype. Ethylene assays were performed as described by Felix et al. (1999) using 1 μM flg22, elf18, or AtPep1.

Seedling Growth Inhibition

Seedlings were germinated on MS agar before transferring two seedlings per well to 24-well plates containing MS or elf18 or flg22 at 1, 10, 30, 60, 100, and 1000 nM concentrations (six wells per concentration). Seedlings were incubated with peptides for 10 d before determination of fresh weight.

Bacterial Infection Assays

The *Pseudomonas syringae* pv *tomato* DC3000 (*Pto* DC3000) or *P. syringae* pv *tabaci* 6605 (*Pta*) strains were grown in overnight culture in Kings B medium supplemented with appropriate antibiotics. Cells were harvested by centrifugation, and pellets were resuspended in sterile water to appropriate OD_{600} (0.2 for *Pto* DC3000 *ΔAvrPto/ΔAvrPto* and *Pto* DC3000 *COR*$^-$; 0.02 for *Pto* DC3000). Immediately prior to spraying, Silwet L-77 was added to bacteria to 0.04% (v/v). Bacteria were sprayed onto leaf surfaces until runoff and plants were maintained at high humidity for 3 d. For syringe inoculation of *P. syringae* pv *tabaci*, bacteria were similarly grown and harvested. Cell pellets were resuspended in sterile water to OD_{600} 0.002 and infiltrated using a needleless syringe into two leaves each of four plants per genotype.

Samples were taken using a cork-borer (20 mm) to cut leaf discs from two leaves per plant and four plants per genotype. Leaf discs were ground in water, diluted, and plated on tryptic soy agar with appropriate selection. Plates were incubated at 28°C and colonies counted 2 d later.

Hyaloperonospora arabidopsidis Inoculation and Growth Assessment

H. arabidopsidis infections were performed as described by Tör et al. (2002). Spores were harvested from infected Ws-*eds1* seedlings 7 d after inoculation, suspended in cold water at a density of 5×10^4 spores/mL, and spray inoculated onto 7-d-old seedlings to the point of runoff. Inoculated seedlings were incubated at high humidity at 18°C for 7 d and were then assessed for sporulation. The growth of the *Hpa* strains Cala2 and Emoy2 was assessed by counting the number of sporangiophores per cotyledon. The reproduction of the *Hpa* strain Emco5 infection was determined by vortexing sporulating seedlings in water and by quantifying spores using a hemocytometer.

Transient Expression in *Nicotiana benthamiana*

Agrobacterium tumefaciens GV3101 strains were grown in L medium supplemented with appropriate antibiotics overnight. Cultures were spun down and resuspended in 10 mM $MgCl_2$ to $OD_{600} = 0.1$. *Agrobacterium* strains carrying Pro35S:*EFR-GFP-His* (*pEarleyGate103*), Pro35S:FLS2-*GFP-His* (*pEarleyGate103*), or Pro35S:BRI1-GFP-His (*pEarleyGate103*) and Pro35S:*SERK-HA*$_3$ (*pGWB14*) were mixed 1:1 and syringe infiltrated into 3-week-old *N. benthamiana* leaves. Samples for protein extraction were harvested 2 d after inoculation. Whole excised leaves were vacuum infiltrated with 100 nM elf18, flg22, or BL. Infiltrated leaves were incubated for 5 min (elf18/flg22) or 3 h (BL) before freezing in liquid nitrogen.

Protein Extraction and Immunoprecipitation in *N. benthamiana*

Leaves were ground in liquid nitrogen and extraction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10% glycerol, 10 mM DTT, 10 mM EDTA, 1 mM NaF, 1 mM $Na_2MoO_4 \cdot 2H_2O$, 1% [w/v] polyvinylpyrrolidone, 1% [v/v] P9599 Protease Inhibitor Cocktail [Sigma-Aldrich], and 1% [v/v] IGEPAL CA-630 [Sigma-Aldrich]) was added at 2 mL/g tissue powder. Samples were clarified by 20 min centrifugation at 4°C and 13,000 rpm. Supernatants (1.5 mL) were adjusted to 2 mg/mL protein and incubated for 4 h at 4°C with 20 μL GFPTrap-A beads (Chromotek). Following incubation, beads were washed four times with TBS containing 0.5% (v/v) IGEPAL CA-630. Thirty microliters of 2× LDS (Invitrogen) was added to the beads, and the beads were heated at 70°C for 15 min.

Protein Extraction and Immunoprecipitation in *Arabidopsis*

Leaves were ground in liquid nitrogen, and extraction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10% glycerol, 5 mM DTT, 2 mM EDTA, 1 mM NaF, 1 mM $Na_2MoO_4 \cdot 2H_2O$, 0.5% [w/v] polyvinylpyrrolidone, 1% [v/v] P9599 Protease Inhibitor Cocktail [Sigma-Aldrich], and 1% [v/v] IGEPAL CA-630 [Sigma-Aldrich]) was added at 2 mL/g tissue powder. Samples were clarified by a 20-min centrifugation at 4°C and 13,000 rpm. Supernatants (45 mL) were adjusted to 3 mg/mL protein and incubated for 4 h at 4°C with 150 μL GFPTrap-A beads (Chromotek). Following incubation, beads were washed four times with TBS containing 0.5% (v/v) IGEPAL CA-630, before adding 2× LDS (Invitrogen) and heating at 70°C for 15 min.

SDS-PAGE and Immunoblotting

SDS-PAGE gels were prepared with either 7.5 or 10% cross-linking. Proteins were loaded and gels run at 100 to 150 V for 1.5 h before electroblotting onto polyvinylidene fluoride membrane (Bio-Rad) at 90 V for 2 h at 4°C. Membranes were rinsed in TBS and blocked for 1 h in 5% nonfat milk in TBS-Tween (0.1% [v/v]). Antibodies were diluted in blocking solution to the following dilutions: anti-GFP (AMS Biotechnology), 1:5000; anti-BAK1, 1:500; anti-HA-horseradish peroxidase (HRP) (Santa Cruz), 1:2000; anti-Flag-AP (Sigma-Aldrich), 1: 5000. Membranes were incubated with primary antibodies overnight. Membranes were washed 3 × 10 min in TBS-T (0.1%) before a 1-h incubation in secondary antibodies anti-rabbit-HRP (Sigma-Aldrich; 1:5000). Chemiluminescent substrate (Lumigen ECL Plus; GE Healthcare) was applied before exposure to film (AGFA CP-BU). For AP-conjugated primary antibodies, membranes were incubated in nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (Roche) until bands were visible.

Antibodies

Polyclonal anti-BAK1 antibodies were generated by immunizing rabbits with a synthetic peptide (DSTSQIENEYPSGPR) derived from the C terminus of BAK1 (Schulze et al., 2010). Antibodies (final bleed) were affinity purified against the peptide (Eurogentec). Anti-FLS2 antibodies have been described elsewhere (Chinchilla et al., 2006).

HPLC and MS

Protein samples were prepared for the MS analysis as described previously (Ntoukakis et al., 2009). LC-MS/MS analysis was performed using a LTQ-Orbitrap mass spectrometer (Thermo Scientific) and a nanoflow-HPLC system (nanoAcquity; Waters) as described previously (Ntoukakis et al., 2009). The entire TAIR9 database was searched (TAIR9 33,596 sequences; 13,487,687 residues) (www.Arabidopsis.org) using Mascot (with the inclusion of sequences of common contaminants, such as keratins and trypsin). Parameters were set for ±5 ppm peptide mass tolerance and allowing for Met oxidation and two missed tryptic cleavages. Carbamidomethylation of Cys residues was specified as a fixed modification, and oxidized Met and phosphorylation of Ser or Thr residues were allowed as variable modifications. Scaffold (v2_06_01; Proteome Software) was used to validate MS/MS-based peptide and protein identifications. Peptide identifications were accepted if they could be established at >95.0% probability as specified by the Peptide Prophet algorithm. Protein identifications were accepted if they could be established at >95.0% probability and contained at least two identified peptides.

Preparation of *Hpa* Extracts

The aerial parts of 48 3- to 4-week-old *Ws-eds1*–infected (*Hpa* Emoy2, 7 d after inoculation) or noninfected plants were harvested and frozen in liquid nitrogen. Twenty milliliters of cold sterile water was added, and the samples were mixed vigorously by vortexing. The suspension was cleared of plant debris by filtering through Miracloth and enriched for heavier particles by centrifugation at 300 rpm for 15 min. The supernatant was removed and the pellet resuspended in 3 mL of sterile water and heated at 95°C for 10 min. These suspensions were used at a concentration of 1:100 to elicit 2-week-old sterile seedlings for 180 min.

Accession Numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under the following accession numbers: SERK1, At1g71830; SERK2, At1g34210; SERK3/BAK1, At4g33430; SERK4/BKK1, At2g13790; SERK5, At2g13800; EFR, At5g20480; FLS2, At5g46330; BRI1, At4g39400; MPK3, At3g45640; MPK4, At4g01370; MPK6, At2g43790; UBQ10, At4g05320; DC1-domain containing protein, At2g17740; CYP81F2, At5g57220; and LRR-RK, At1g51890.

Supplemental Data

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

Supplemental Figure 1. Specificity of the Anti-BAK1 Antibodies.

Supplemental Figure 2. Coimmunoprecipitation of BRI1 and BAK1 in *N. benthamiana*.

Supplemental Figure 3. SERK-HA Proteins Do Not Adhere to GFP Trap Beads Nonspecifically.

Supplemental Figure 4. PAMP-Induced ROS Burst and Seedling Growth Inhibition of Single *serk* Mutants.

Supplemental Figure 5. *serk1* or *serk2* Mutations Do Not Enhance the Phenotype of bak1-5 Seedlings.

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