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Arabidopsis WRKY70 Is Required for Full *RPP4*-Mediated Disease Resistance and Basal Defense Against *Hyaloperonospora parasitica*

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AtWRKY70, **encoding a WRKY transcription factor, is co-expressed with a set of *Arabidopsis* genes that share a pattern of *RPP4*- and *RPP7*-dependent late upregulation in response to *Hyaloperonospora parasitica* infection (*LURP*) genes. We show that *AtWRKY70* is required for both full *RPP4*-mediated resistance and basal defense against *H. parasitica*. These two defense pathways are related to each other, because they require *PAD4* and salicylic acid (SA). *RPP7* function, which is independent from *PAD4* and SA, is not affected by insertions in *AtWRKY70*. Although *AtWRKY70* is required for *RPP4*-resistance, it appears not to contribute significantly to *RPP4*-triggered cell death. Furthermore, our data indicate that AtWRKY70 functions downstream of defense-associated reactive oxygen intermediates and SA. Constitutive and *RPP4*-induced transcript levels of two other *LURP* genes are reduced in *AtWRKY70* T-DNA mutants, indicating a direct or indirect role for AtWRKY70 in their regulation. We propose that AtWRKY70 is a component of a basal defense mechanism that is boosted by engagement of either *RPP4* or *RPP7* and is required for *RPP4*-mediated resistance.**

Additional keywords: disease resistance.

Recognition of biotrophic pathogens triggers complex defense programs resulting in extensive transcriptional reprogramming (Eulgem 2005; Katagiri 2004; Somssich and Hahbrock 1998). Genes upregulated during plant immune responses can have important roles for disease resistance (Bartsch et al. 2006; Ramonell et al. 2005; Rowland et al. 2005; Veronese et al. 2006). However, molecular mechanisms that translate recognition of pathogens into appropriate transcriptional outputs are still poorly understood.

At least two mechanisms of pathogen recognition are used by plants. Perception of chemical signatures ubiquitously present in large classes of pathogens can activate basal defense, which restricts growth of a wide range of pathogens (Chisholm et al. 2006; Nürnberger et al. 2004). In addition, disease resistance (*R*) genes direct detection of distinct pathogen races by genetically interacting with pathogen-derived avirulence (*avr*) genes. R protein engagement triggers strong "gene-for-gene" resistance, resulting in incompatible plant–pathogen interactions (plant resistant, pathogen avirulent) (Dangl and Jones 2001; Flor 1971). A hallmark of *R*-mediated resistance is the hypersensitive response (HR), a programmed death of plant cells at infection sites. Absence of *R*-mediated recognition gives rise to compatible interactions and the development of disease symptoms (plant susceptible, pathogen virulent) (Hammond-Kosack and Parker 2003). Basal defense responses, however, can be active during compatible interactions (Glazebrook 2001; Glazebrook et al. 1996).

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Microarray studies suggested that differences between gene-for-gene resistance and basal defense in *Arabidopsis thaliana* are quantitative rather that qualitative (Eulgem et al. 2004; Maleck et al. 2000; Navarro et al. 2004; Tao et al. 2003). Katagiri and coworkers proposed that *R*-mediated pathogen recognition can boost basal defense reactions, resulting in accelerated and more intense responses (Katagiri 2004; Tao et al. 2003). This quantitative signaling mechanism may involve coordinated production of the reactive oxygen intermediates (ROI) O_2^-, H_2O_2, and NO as well as salicylic acid (SA) (Delledonne et al. 2002; Durner and Klessig 1999; Torres and Dangl 2005). SA triggers downstream signaling processes activating defense-associated genes as well as systemic acquired resistance (SAR) (Klessig et al. 2000; Maleck et al. 2000; Schenk et al. 2000).

In *Arabidopsis*, both basal defense and SAR are dependent on SA as well as NPR1, a nuclear transported transcriptional cofactor (Cao et al. 1994, 1997; Kinkema et al. 2000; Mou et al. 2003; Ryals et al. 1997). While application of SA (White 1979) or its functional analog 2,6-dichloroisonicotinic acid (INA) (Kessmann et al. 1993; Uknes et al. 1992) effectively induce SAR and basal defense, blocking of SA accumulation by mutations in *PAD4* or overexpression of a bacterial SA hydroxylase gene (*nahG*) abolish these defense responses (Delaney et al. 1994; Jirage et al. 1999; Nawrath et al. 2002; Wildermuth et al. 2001). Mutations in *NPR1* also block basal defense and SAR, including many SA- or INA-inducible responses, indicating a role of NPR1 downstream from SA (Dong 2004).

Several *Arabidopsis* transcription factors, including members of the large family of WRKYs, have been implicated in defense signaling (Dong et al. 2003; Eulgem et al. 2000; Maleck et al. 2000; Ulker and Somssich 2004; Wu et al. 2005). The defining feature of WRKYs is the conserved DNA binding domain of approximately 60 amino acids containing the nearly invariant stretch WRKYGQK followed by a unique zinc-finger pattern of Cys and His residues (Rushton et al. 1996). WRKYs were subdivided into three groups (Eulgem et al. 2000). Members of group I have two WRKY domains, whereas members of groups II and III have one WRKY domain. Group III WRKY

domains contain a $Cx_7CX_{23}HXC$ pattern of zinc ligands which is distinct from the $Cx_{4-5}CX_{22-23}HXH$ zinc finger pattern of group I and II WRKY domains. Mutations in either the WRKYGQK or the zinc finger motif of WRKY domains compromised their DNA binding ability (Maeo et al. 2001). Most WRKYs seem to interact specifically with a DNA motif termed W box (TTGACC/T).

The nearly ubiquitous presence of W boxes in promoters of defense-associated genes strongly suggests a broad role of WRKY factors in resistance to pathogens (Chen et al. 2002; Dong et al. 2003; Eulgem et al. 2004; Maleck et al. 2000; Navarro et al. 2004; Ramonell et al. 2002). Overexpression of defined *Arabidopsis WRKY* (*AtWRKY*) genes altered resistance to pathogenic bacteria or fungi (Asai 2002; Chen and Chen 2002; Li et al. 2004). Silencing of three separate *WRKY* genes in tobacco reduced resistance to *Tobacco mosaic virus* mediated by the *R* gene *N* (Liu et al. 2004). The *Arabidopsis* gene *RRS1-R* encodes an atypical group III WRKY (AtWRKY52) with structural features of R proteins that confers resistance to several strains of *Ralstonia solanacearum* (Deslandes et al. 2002, 2003). A recent study revealed complex functions in disease resistance for the structurally related AtWRKY18, AtWRKY40, and AtWRKY60 proteins (Xu et al. 2006). These proteins were shown to have partially redundant roles in activating defense to the fungal necrotroph *Botrytis cinerea* and repressing basal resistance to a virulent strain of the bacterial hemibiotroph *Pseudomonas syringae*.

Complex roles also were demonstrated for *AtWRKY70*, encoding a group III WRKY protein. Although *AtWRKY70* contributes to basal resistance to the virulent biotrophic fungus *Erysiphe chicoracearum*, it represses defenses to the fungal and bacterial necrotrophs *Alternaria brassicicola* as well as *Erwinia carotovora,* respectively (Li et al. 2004, 2006). Unlike resistance to biotrophs, which frequently is mediated by SA, resistance to necrotrophs has been associated with jasmonic acid (JA) (Glazebrook 2001). Multiple studies have demonstrated antagonistic crosstalk between SA and JA signaling (Glazebrook et al. 2003; Kunkel and Brooks 2002; Petersen et al. 2000; Spoel et al. 2003). AtWRKY70 serves as an activator of SA-inducible pathogenesis-related (*PR*) genes and a repressor of the JA-inducible gene *PDF2-1*; therefore, it was suggested to have a role in determining the balance between SA and JA signaling (Li et al. 2004, 2006). Furthermore, epistasis analyses indicated that AtWRKY70 operates downstream from defense-associated SA accumulation as well as downstream or independent from NPR1.

We examined interactions between *Arabidopsis* and the obligate biotrophic oomycete pathogen *Hyaloperonospora parasitica* (Holub et al. 1994; Slusarenko and Schlaich 2003). Different *H. parasitica* isolates are recognized by distinct *Arabidopsis R* genes designated as *RPP* (recognition of *Hyaloperonospora parasitica*) (Holub et al. 1995). Using Affymetrix DNA chips we identified a set of coexpressed genes sharing a pattern of late upregulation in response to *H. parasitica* recognition (*LURP*) that contain the genes initially designated cluster II (Eulgem et al. 2004; Eulgem et al. in press). Elevated levels of *LURP* transcripts coincide with the appearance of HR. Interestingly, *LURP* genes are upregulated following stimulation of RPP4 or RPP7 which mediate resistance to the *H. parasitica* isolates Emoy2 (*Hp*Emoy2) or Hiks1 (*Hp*Hiks1), respectively, by genetically separable signaling mechanisms (McDowell et al. 2000; van der Biezen et al. 2002). *LURP* transcript levels typically exhibit a continuous steep increase over the first 48 h postinfection (hpi) (Fig. 1) (Eulgem et al. 2004; Eulgem et al. in press). The accumulation of *LURP* transcripts is delayed or attenuated in *H. parasitica*-susceptible lines lacking *RPP4* or *RPP7* function, suggesting a role of *LURP* genes in defense reactions triggered by these two RPP proteins.

AtWRKY70 is included in the *LURP* set (Fig. 1) (Eulgem et al. 2004, represented by probe set "14201_at" in Supplemental Table 5). Here, we demonstrate that *AtWRKY70* is an important component of *RPP4*-mediated resistance and basal defense to *H. parasitica*. Our data further suggest that AtWRKY70 operates downstream from ROI production and SA accumulation in *H. parasitica*-induced defense signaling. Moreover, we show that AtWRKY70 controls transcript levels of at least two other *LURP* genes, suggesting a role for AtWRKY70 in transcriptional reprogramming required for resistance to *H. parasitica*. Our results extend previous findings (Li et al. 2004, 2006) by providing additional evidence for roles of *AtWRKY70* in resistance to biotrophs and by establishing *AtWRKY70* as a genetic component of *RPP4*-mediated and basal resistance to the oomycete pathogen *H. parasitica*.

RESULTS

The *wrky70.1* and *wrky70.3* T-DNA mutants exhibit substantially reduced *AtWRKY70* transcript levels.

Two independent Col-0 mutants with insertions in *AtWRKY70* were found in sequence-indexed T-DNA mutant collections (Alonso et al. 2003; Sessions et al. 2002). SALK_025198 (*wrky70-1*) has an insertion in the first exon and SAIL_720_E01 (*wrky70-3*) has an insertion in the second intron of *AtWRKY70* (Fig. 2A). Homozygous T3 or T4 individuals for the respective insertions were selected by polymerase chain reaction (PCR)-based genotyping (Alonso et al. 2003) and selfed. Their progeny were used for experiments. The genomic location of each insertion was confirmed by sequencing. Shortly before our manuscript was submitted, a paper by Li and associates was published describing SALK_025198 and a third *wrky70* T-DNA mutant (*wrky70-2*, GABI_324D11) (Li et al. 2006). We adhered to their nomenclature designating SALK_025198 as *wrky70-1*. Accordingly, we designated our new *wrky70* SAIL allele as *wrky70-3*.

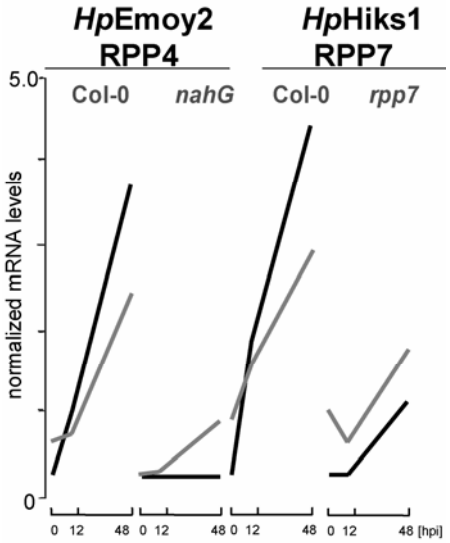

Fig. 1. *AtWRKY70* exhibits a *LURP*-type expression profile. Levels of *AtWRKY70* transcript (black) as well as average levels of late upregulated in response to *Hyaloperonospora parasitica* infection (*LURP*) transcripts (gray) in Col-0, *nahG,* or *rpp7* plants at the indicated time points postinfection with *Hp*Emoy2 or *Hp*Hiks1. The shown data are derived from a study using Affymetrix Arabidopsis genome arrays (Eulgem et al. 2004). The gray graph represents the weighted average profile of 38 *LURP* genes as defined previously. Similar results were obtained in an independent study with an Affymetrix custom *Arabidopsis* whole-genome array.

Cloned full-length *AtWRKY70* cDNAs comprise 916 to 1,119 bp (accession numbers AY142566, AF421157, AY087389, and AY039933). Consistent with this, RNA blotting with an *AtWRKY70* specific probe resulted in a strong approximately 1,100-nucleotide (nt) band in Col-0 plants likely representing the *AtWRKY70* transcript (Fig. 2B). As expected, based on our transcriptional profiling data (Fig. 1) (Eulgem et al. 2004; T. Eulgem and J. Dangl, *unpublished*), levels of the 1,100-nt transcript were upregulated in Col-0 after infection with *Hp*Emoy2. Both *wrky70* alleles exhibited a faint band of approximately 1,100 nt in length. The *wrky70-3* insertion resides in an intron and can be removed from transcripts by splicing. Hence, in this mutant, the weak approximately 1,100-nt band may be due partially to residual levels of wild-type *AtWRKY70* transcript. The *wrky70-1* insertion, however, resides in an exon and cannot be eliminated by splicing. Therefore, in this allele, the faint approximately 1,100-nt band is unlikely to be due to residual levels of wild-type *AtWRKY70* transcript and, rather, results from transcripts of one or several *WRKY* genes closely related to *AtWRKY70*. Five other group III WRKYs have transcripts with sizes similar to those of the *AtWRKY70* transcript, ranging from 973 to 1,150 nt (TAIR accession numbers 3709717, 1009062086, 1009057659, and 1009063399) that may have weakly hybridized with the probe used in our Northern blot analysis.

In *wrky70-3*, an additional band representing an approximately 500-nt transcript was strongly induced at 24 hpi. Its levels remained nearly constant between 24 and 48 hpi. The *wrky70-3* insertion is located between positions 487 and 488 of the coding region of full-length *AtWRKY70* cDNAs, likely explaining the truncated mRNA. The protein potentially encoded by this putative *AtWRKY70* transcript would lack the zinc finger-like motif of its DNA binding domain and is unlikely to constitute a functional WRKY transcription factor (Fig. 2C). No obvious developmental or morphological phenotypes were observed in either *wrky70* mutant. In summary, the *wrky70-1* and *wrky70-3* mutants have either severely reduced or completely abolished levels of wild-type *AtWRKY70* transcript and a truncated transcript, respectively. Hence, function of *AtWRKY70* is likely to be substantially compromised in both mutants.

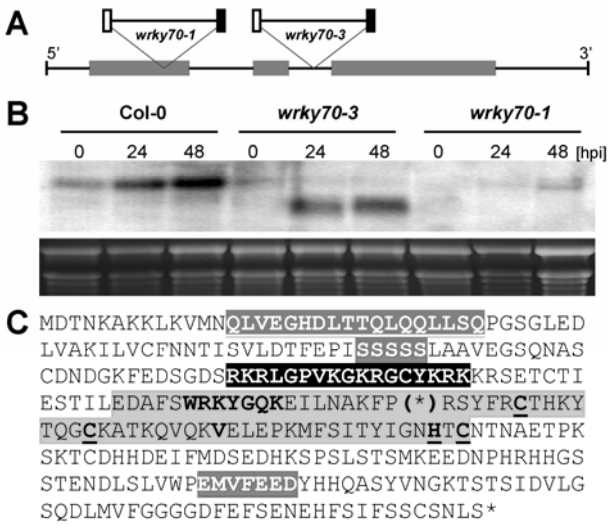

Fig. 2. Two T-DNA insertion lines with altered *AtWRKY70* expression. **A,** Cartoon illustrating position and orientation of T-DNA insertions in *AtWRKY70*. T-DNA boarders are represented by open (right boarder) and black (left boarder) boxes. Mutant *wrky70.1* (SALK_025198) has an insertion in the first exon and *wrky70.3* (SAIL_720_E01) has an insertion in the second intron. Gray boxes signify exons, lines are introns. **B,** RNA gel blot analysis of *AtWRKY70* expression in wild-type (Col-0) and T-DNA insertion mutants 0, 24, and 48 h after infection with *Hp*Emoy2 (5×10^4 spores/ml suspension). The ethidium bromide-stained RNA gel was photographed as loading control. **C,** Primary structure of AtWRKY70 based on full-length cDNA sequences and TAIR gene model AT3G56400.1. The WRKY DNA binding domain is highlighted in light gray. The "WRKYGQK motif" that is invariant in most WRKY domains is in bold. Cysteine and histidine residues of the zinc-finger motif conserved in group III WRKY domains are underlined. A putative bipartite nuclear localization signal detected by Prosite is highlighted in black and printed in white. An N-terminal glutamine-rich region and a C-terminal acidic region that may constitute transactivation domains are highlighted in dark gray and printed in white. A truncated AtWRKY70 transcript in *wrky70-3* putatively encodes a protein without the zinc-finger motif reaching up to "*".

Mutations in *AtWRKY70* compromise function of *RPP4* but not *RPP7*.

We next tested whether these mutations in *AtWRKY70* affect resistance to *Hp*Emoy2 and *Hp*Hiks1 (Fig. 3). In Col-0 cotely-

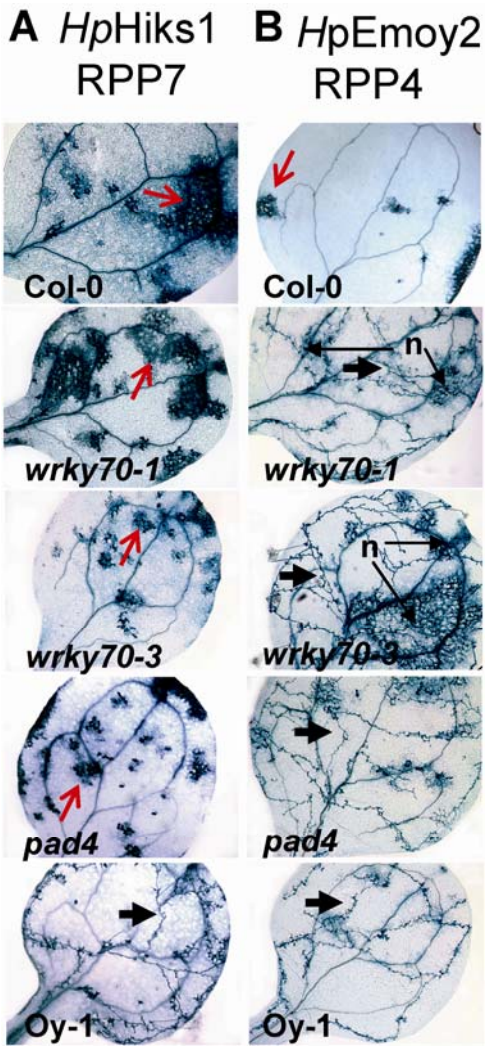

Fig. 3. The *wrky70* T-DNA mutants are compromised in resistance to *Hp*Emoy2, but not *Hp*Hiks1. Col-0 (resistant; wild-type), Oy-1 (susceptible control), and mutants were stained with Trypan Blue 7 days after spraying with 5×10^5 spores of *Hyaloperonospora parasitica* per milliliter to visualize hyphal growth (bold black arrows) and cell death (hypersensitive response [HR]) (red arrows). **A,** The *wrky70* mutant lines infected with *Hp*Hiks1 show discrete HR sites similar to Col-0 and resistant *pad4* plants. Oy-1 exhibits heavy hyphal growth. **B,** After infection with *Hp*Emoy2, hyphae grow past the penetration site but are surrounded by a trail of necrotic plant cells (n; thin arrows) in *wrky70* mutants. Col-0 has discrete HR sites. Oy-1 ecotype and the *pad4* mutant exhibit heavy hyphal growth with minimal associated cell death. **A** and **B,** Three independent repetitions (each including at least 20 seedlings per plant genotype) gave similar results.

dons, which express both *RPP4* and *RPP7*, the two *H. parasitica* isolates triggered HR and were unable to form extended hyphae. In contrast, dense networks of *Hp*Hiks1 and *Hp*Emoy2 hyphae, and no HR sites, developed in cotelydons of the *Arabidopsis* ecotype Oy-1, which lacks both *RPP4* and *RPP7* (Holub et al. 1994). As previously described (Glazebrook et al. 1997; van der Biezen et al. 2002), the *pad4* mutant exhibits wild-type HR in response to *Hp*Hiks1, but supports prolific hyphal growth when challenged with *Hp*Emoy2. Development of HR and disease resistance to *Hp*Hiks1 were unaffected in *wrky70.1* and *wrky70.3*. However, we observed pronounced growth of *Hp*Emoy2 hyphae in cotelydons of these mutants. *Hp*Emoy2 hyphae in *wrky70.1* and *wrky70.3* frequently were surrounded by necrotic plant cells. Such trailing necrosis is believed to be the result of weak *R* gene activity that is insufficient to halt pathogen growth (Torres et al. 2002). Thus, our data demonstrate a differential role for *AtWRKY70* in gene-for-gene resistance to *H. parasitica*. *AtWRKY70* is required for full *RPP4* resistance, but not for *RPP7* resistance to *H. parasitica*. Furthermore, the induction of *RPP4*-mediated cell death is not fully blocked in *wrky70* mutants, suggesting that *AtWRKY70* does not contribute significantly to HR.

Mutations in *AtWRKY70* do not affect *RPP4*-mediated ROI production.

R-triggered HR typically is associated with the accumulation of ROI (Lamb and Dixon 1997; Torres and Dangl 2005). In cell cultures, ROI production is one of the first physiological reactions observed after pathogen recognition and occurs within minutes after application of defense-related stimuli (Jabs et al. 1997; Levine et al. 1994; Piedras et al. 1998). In intact plant tissue, *R*-dependent induction of ROIs is detectable after 4 to 24 h, depending on the respective interaction (Eulgem et al. in press; Shapiro and Zhang 2001; Torres et al. 2002). To determine the functional relationship between *AtWRKY70* and *RPP4*-dependent ROI production, we stained *Hp*Emoy2-infected seedlings with 3,3′-diaminobenzidine (DAB) (Fig. 4). DAB staining results in the deposition of a brownish precipitate in the presence of H_2O_2 (Torres et al. 2002). Cotyledons of Col-0 seedlings typically exhibited DAB staining by 24 hpi with *Hp*Emoy2. As expected, DAB staining was completely absent in cotyledons from Oy-1 seedlings. Both *wrky70.1* and *wrky70.3* exhibited DAB staining to an extent similar to that observed in Col-0 at 24 hpi with *Hp*Emoy2. Thus, AtWRKY70 appears not to be required for *RPP4*-mediated ROI production, and is likely to operate either independently or downstream of the oxidative burst.

Mutations in *AtWRKY70* compromise basal defense.

Many mutations that affect *R*-mediated resistance also reduce basal defense responses, resulting in enhanced susceptibility to virulent pathogens (Glazebrook et al. 1996). Therefore, we tested whether basal defense to the virulent *H. parasitica* isolate Noco2 is affected in *wrky70* mutants (Fig. 5). *Hp*Noco2 is not recognized by any Col-0 *R* gene, and its interaction with Col-0 is compatible. After spray inoculation with 3×10^4 *Hp*Noco2 spores, we observed a moderate level of spore formation on Col-0 plants. The *pad4* mutant, which is known to be deficient in basal defense responses (Jirage et al. 1999), exhibited pronounced hypersusceptibility, resulting in a nearly fourfold increase of spores per fresh weight. In *wrky70.1* and *wrky70.3*, we observed a two- to threefold increase of spore formation relative to Col-0. Thus, *wrky70.1* and *wrky70.3* also exhibit hypersusceptibility to *Hp*Noco2, albeit to a lesser degree than *pad4*. These results prove that *AtWRKY70* has a role in basal defense against *H. parasitica*.

Mutations in *AtWRKY70* reduce INA-mediated resistance to *H. parasitica*.

INA is a potent inducer of multiple SA-dependent defense responses, such as basal defense, SAR, and *PR* gene expression (Kessmann et al. 1994). To determine the functional relationship of *AtWRKY70* to SA or INA signaling, we tested effects of mutations in *AtWRKY70* on INA-mediated resistance to *Hp*Noco2 (Fig. 6). Col-0 and mutant plants were treated with INA and, 48 h later, spray inoculated with *Hp*Noco2 spores. Although untreated Col-0 plants were susceptible to *Hp*Noco2, INA-treated Col-0 plants exhibited strong resistance to *Hp*Noco2, not permitting formation of any sporangiophores. As expected, INA did not trigger *Hp*Noco2 resistance in the *npr1* mutant, which is known to be compromised in signaling steps downstream from SA or INA perception (Dong 2004). In the *pad4* mutant, which is deficient in defense-associated SA accumulation, INA-mediated resistance was not significantly affected. In *wrky70.1* and *wrky70.3*, INA-mediated resistance to *Hp*Noco2 was moderately reduced, indicating that *AtWRKY70* contributes to defense mechanisms triggered by INA and likely operates downstream from INA and SA perception.

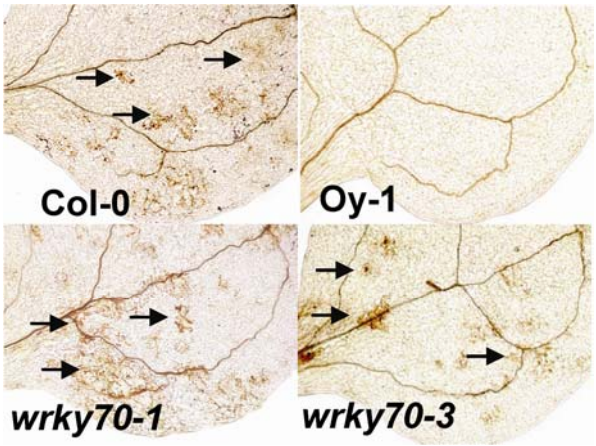

Fig. 4. *AtWRKY70* is not required for *RPP4*-mediated reactive oxygen intermediates production. Shown are cotelydons of 2-week-old wild-type and mutant seedlings stained with 3,3-diaminibenzidine (DAB) 24 h postinfection with *Hp*Emoy2 (5×10^4 spores/ml). Arrows indicate localized DAB staining. Two independent repetitions (each including at least 10 seedlings per plant genotype) gave similar results.

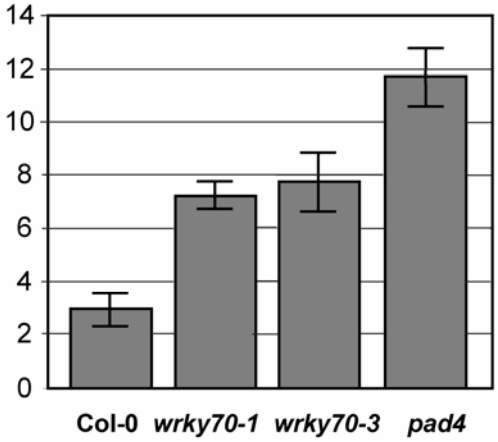

Fig. 5. The *wrky70* mutants are compromised in basal defense to *Hp*Noco2. Plants were sprayed with virulent *Hp*Noco2 (3×10^4 spores/ml). Spores were counted 7 days postinfection. The Mann-Whitney U test detected significant differences ($P < 0.05$) between spore counts from Col-0 and all tested mutants. Mean and standard error were calculated from three independent experiments.

AtWRKY70 regulates expression of *CaBP22* and *LURP1*.

CaBP22 (At2g41090) and *LURP1* (At2g14560) belong to the set of *LURP* genes and are coexpressed with *AtWRKY70* after *Hp*Hiks1 and *Hp*Emoy2 recognition. The profiles of normalized *CaBP22* or *LURP1* transcript levels exhibit a high correlation with those of *AtWRKY70* (Pearson correlation > 0.90) (Eulgem et al. 2004; Eulgem et al. in press). *AtWRKY70* encodes a transcription factor. High levels of its expression correlate with high levels of *CaBP22* and *LURP1* expression. Thus, AtWRKY70 may have a role in promoting *CaBP22* and *LURP1* transcription. Therefore, we tested, by RNA blotting, whether steady state levels of *CaBP22* and *LURP1* transcripts are affected in *wrky70* mutants (Fig. 7). Both constitutive and *Hp*Emoy2-induced levels of *LURP1* and *CaBP22* transcript at 0, 24, and 48 hpi were reduced in *wrky70.1* and *wrky70.3* compared with Col-0 plants. Thus, AtWRKY70 appears to directly or indirectly contribute to constitutive and *Hp*Emoy2-triggered expression of *CaBP22* and *LURP1*.

DISCUSSION

Using T-DNA insertion mutants, we demonstrated a role of *AtWRKY70* in gene-for-gene disease resistance and basal defense to the oomycete pathogen *H. parasitica*. In all experiments we performed, both *wrky70* alleles exhibited qualitatively and quantitatively nearly identical phenotypes. This indicates that these phenotypes were caused by the insertions in *AtWRKY70* and not by mutations in other genes. Effects of *wrky70* mutations on *H. parasitica* resistance were partial. *RPP4*-mediated resistance to *Hp*Emoy2 was reduced, but not fully abolished, in *wrky70* mutants. Although free *Hp*Emoy2 hyphae developed in Oy1, which completely lacks *RPP4* function, in *wrky70* mutants they typically were accompanied by trailing necrosis, indicating residual *RPP4* activity. Similarly, basal defense and INA-mediated resistance were reduced only moderately in *wrky70* mutants.

There are several possible explanations for the partial nature of *wrky70-1* and *wrky70-3* phenotypes. The simplest is that residual *AtWRKY70* activity in *wrky70-3,* which may not be a complete null allele, prevents more drastic phenotypes. Furthermore, AtWRKY70 is one of 13 structurally related members of group III of AtWRKYs (Kalde et al. 2003). Nearly all group III *AtWRKY* genes are transcriptionally upregulated in response to SA and *Hp*Emoy2, suggesting common roles in SA-mediated resistance to *H. parasitica* (Kalde et al. 2003).

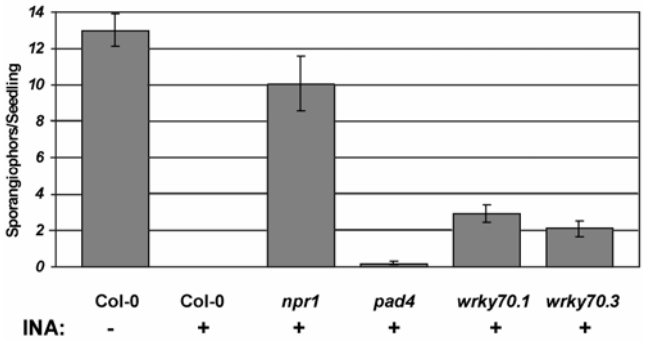

Fig. 6. *AtWRKY70* mutants exhibit a partial reduction in 2,6-dichloroisonicotinic acid (INA)-mediated resistance to *Hp*Noco2. Two-week-old seedlings were sprayed with 0.33 mM INA and incubated for 2 days before spraying with *Hp*Noco2 (3×10^4 spores/ml). Spores were counted 7 days after *Hp*Noco2 infection. Significantly more sporangiophores were counted on the *wrky70* mutants than Col-0 after INA treatment (Mann-Whitney U test, $P < 0.05$). Mean and standard error were calculated from two independent experiments (each including at least 30 individuals per plant genotype).

Numerous genes encoding group I and group II WRKYs also exhibit increased transcript levels in response to SA (Dong et al. 2003). Therefore, additional *WRKY* genes may compensate partially for abolished or reduced *AtWRKY70* activity and confer a certain degree of resistance to *H. parasitica* in *wrky70* mutants. Finally, pathway branches that are independent of AtWRKY70 and other WRKYs might control defense functions providing partial protection in *wrky70* mutants. One such pathway branch is likely to involve the Whirly-type transcription factor AtWhy1 which contributes to SA-mediated *RPP4* resistance to *Hp*Emoy2 (Desveaux et al. 2004).

Interestingly, we found only *RPP4*-mediated resistance to be affected in *wrky70* mutants. No effect of *wrky70* mutations on *RPP7* function could be detected by our assays. The differential significance of *AtWRKY70* for *RPP4*- and *RPP7*-mediated resistance may reflect dependency of these *R* genes on different defense-signaling mechanisms. Although *RPP4* resistance is dependent on SA signaling, *RPP7* resistance is independent of SA and most other known defense-signaling components (McDowell et al. 2000). We also demonstrated that *AtWRKY70* is not required for *RPP4*-mediated ROI production. Full INA-mediated resistance to virulent *Hp*Noco2, however, requires *AtWRKY70*. These data suggest a role of AtWRKY70 downstream from the oxidative burst and SA or INA perception. This interpretation is consistent with reports by Li and associates (2004, 2006) placing AtWRKY70 downstream from SA accumulation.

In addition to *RPP4*-mediated resistance, basal defense to a virulent *H. parasitica* isolate is compromised in *wrky70* mutants. Basal defense to *H. parasitica* is dependent on SA, *PAD4*, and additional defense regulators (Glazebrook 2001; van der Biezen et al. 2002). Thus, our data are consistent with the model proposed by Katagiri (2004), which suggests that at least some *R* genes boost functions overlapping with the basal defense system, resulting in accelerated and more intense transcriptional responses. For transcripts controlled by such a quantitative mechanism, this model predicts three different scenarios after infection with a pathogen avirulent on the respective wild-type host: i) intense and rapid induction in the presence of intact *R*-mediated recognition and basal defense, ii) delayed and attenuated upregulation in the absence of *R*-mediated recognition but intact basal defense, and iii) strongly reduced levels in the absence of basal defense.

The transcript profiles of *AtWRKY70* and other *LURP* genes exhibit exactly those features (Fig. 1). *RPP4*- and *RPP7*-mediated recognition causes a steep increase of their transcript levels in response to infection with *Hp*Emoy2 and *Hp*Hiks1, respectively. Lack of *R*-mediated recognition in *rpp7* plants results in a clear delay and partial attenuation of *Hp*Hiks1-induced

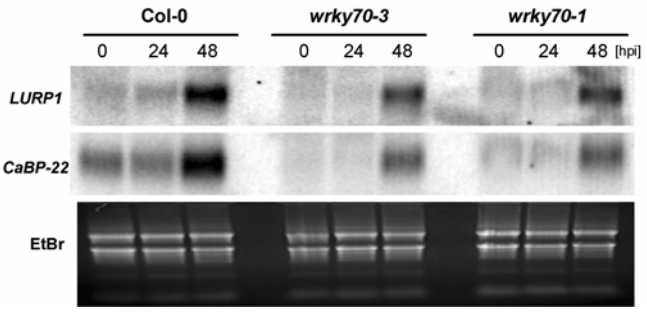

Fig. 7. *AtWRKY70* controls transcript levels of two other late upregulated in response to *Hyaloperonospora parasitica* infection (*LURP*) genes. RNA gel blot analysis of the expression of *LURP* genes (*LURP1 and CaBP22)* in Col-0 (wild-type) and *wrky70* mutants 0, 24, and 48 h postinfection with *Hp*Emoy2 (5×10^4 spores/ml). The ethidium bromide (EtBr)-stained RNA gel was photographed as loading control.

LURP transcript accumulation. Blocking of *RPP4* function and basal defense in *nahG* plants nearly completely abolishes *Hp*Emoy2-induced transcript accumulation.

Based on this characteristic transcript pattern, we propose that *H. parasitica*-induced upregulation of *AtWRKY70* and *LURP* transcripts is part of a complex system that incorporates basal defense elements boosted by *RPP4 and RPP7* (Fig. 8). Increased activity of AtWRKY70 and other *LURP* products as a result of their transcriptional upregulation may lead to the activation of defense reactions containing growth of *Hp*Emoy2. However, in *wrky70* mutants, resistance to *Hp*Hiks1 is not affected. *RPP7* may trigger additional defense mechanisms independent of *AtWRKY70*, which are sufficient to fully abolish growth of the pathogen. By contrast, *RPP4* apparently does not trigger these putative *AtWRKY70*-independent mechanisms, or activates them to a lower extent insufficient to abort pathogen growth. As a result *RPP4*, but not *RPP7*, at least partially depends on *AtWRKY70*-mediated defenses.

Transcript levels of two *LURP*s, *LURP1* and *CaBP22*, are reduced in *wrky70* mutants, indicating a direct or indirect role of AtWRKY70 in regulating transcription of these genes. Such a role in transcriptional upregulation is consistent with the primary structure of AtWRKY70 (Fig. 2C). We inspected *LURP* upstream sequences for putative WRKY-binding sites (W boxes; TTGACC/T). Although two such motifs are located within 1,000 bp upstream from the *LURP1* coding region, no canonical W box motifs are present in the entire 1,230-bp intergenic region upstream from *CaBP22*, or in the transcribed region of this gene. Promoters targeted by WRKY factors tend to contain clusters of multiple W boxes (Eulgem et al. 1999; Turck et al. 2004; Yu et al. 2001). Furthermore, promoters of gene sets putatively coregulated by WRKYs were found to be statistically enriched for W boxes or related motifs (Chen et al. 2002; Dong et al. 2003; Eulgem et al. 2004; Maleck et al. 2000). However, we did not observe any significant enrichment of W box motifs in promoters of the *LURP* set. In summary, our results suggest that AtWRKY70 does not directly interact with promoter elements of *CaBP22* as well as most other *LURP* genes and has an indirect role in regulating these genes. Alternatively, AtWRKY70 may interact with a DNA sequence distinct from canonical W boxes in *LURP* promoters. No studies addressing the DNA-binding properties of AtWRKY70 have been published yet. Although most WRKYs appear to bind to W boxes, some exceptions have been reported (Sun et al. 2003; Xu et al. 2006).

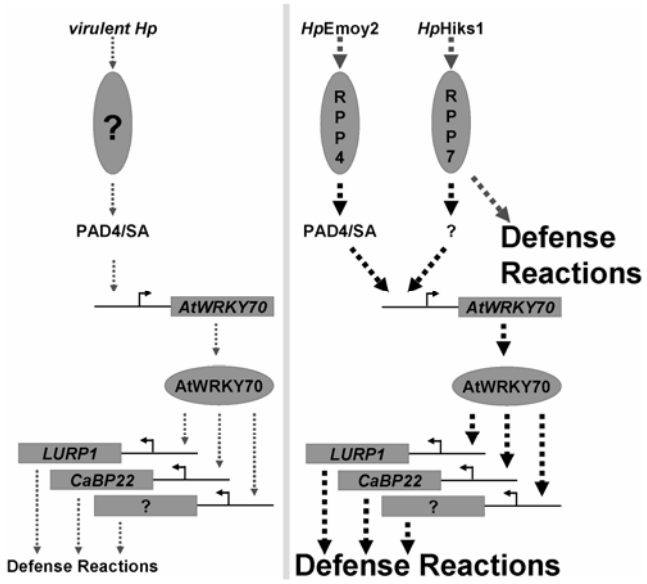

Fig. 8. Model illustrating a role of AtWRKY70 as a regulator of basal defense responses to *Hyaloperonospora parasitica* (*Hp*). Infection with virulent *H. parasitica* weakly upregulates *AtWRKY70* transcript levels, resulting in a moderate increase of AtWRKY70 activity and moderate activation of defense reactions. *RPP4*- and *RPP7*-mediated recognition of *H. parasitica* boosts (bold black arrows) this *AtWRKY70*-dependent defense mechanism. *RPP7* triggers additional defense reactions and is not dependent on *AtWRKY70*. Ovals represent proteins, rectangles represent genes, and dashed arrows represent direct or indirect activating interactions.

AtWRKY70 mutants now have been shown to be compromised in resistance to two distinct eukaryotic biotrophs, the oomycete *H. parasitica* and the fungus *Erysiphe chicoracearum* (Li et al. 2006; this study). These two pathogens are phylogenetically divergent (stramenopile and fungi) and exhibit different lifestyles. Although *H. parasitica* forms an intercellular mycelium in host tissue, *E. chicoracearum* mycelia grow on leaf surfaces. We surveyed microarray data from public data repositories using Genevestigator (Zimmermann et al. 2004) and found *AtWRKY70* transcripts to be upregulated in response to a variety of stimuli that trigger defenses against biotrophs. For example, elevated *AtWRKY70* transcript levels were triggered under several SAR-inducing conditions as well as after treatment with pathogen-associated molecular patterns or the oomycete *Phytophthora infestans*.

These observations, together with our experimental data, suggest a broad role of AtWRKY70 as a positive regulator of resistance to a variety of biotrophs. Combined with other regulatory components, AtWRKY70 is likely to be part of defense mechanisms triggered in response to multiple stimuli and effective against many pathogens. *CaBP22*, *LURP1*, and possibly other *LURP* genes are directly or indirectly controlled by AtWRKY70. Additional transcription factors are likely to participate in their regulation. The *wrky70* T-DNA mutants will be valuable tools for the future dissection of AtWRKY70-dependent defense mechanisms and will allow determination of the full set of its direct and indirect target genes by transcriptional profiling and other methods.

MATERIALS AND METHODS

Plants and growth conditions.

Arabidopsis thaliana ecotypes and mutants were grown on soil under fluorescent lights (10-h day and 14-h night, 21ºC, 100 μEinstein/m^2s). All plants used in this study are in the Col-0 ecotype background. The mutants *pad4-1* (Glazebrook et al. 1997) and *npr1-1* (Cao et al. 1994) have been described.

Selection of *wrky70* T-DNA mutants.

Two *AtWRKY70* T-DNA mutants, SALK_025198 and SAIL_720_E01, were obtained from ABRC and Syngenta, respectively. Seed were grown on soil for 5 weeks; then, genomic DNA was extracted. Homozygous lines were selected by PCR using a T-DNA-specific primer and a pair of gene-specific primers flanking the insertion site, as described previously (Alonso et al. 2003). For SALK_025198 (*wrky70.1*), a T-DNA-specific primer (LBb1 5′-GCGTGGACCGCTTGCTGC AACT-3′) and two gene-specific primers (FP 5′-AGCTCAAC CTTCTGGACTTGC-3′and RP 5′-ATGAACCAACTCGTTG AAGGC-3′) were used. For SAIL_720_E01 (*wrky70.3*), a T-DNA-specific primer (LB2 5′-GCTTCCTATTATATCTTCCC AAATTACCAATACA -3′) and two gene-specific primers (FP 5′-CTGTTATGGTTAGTCACAAACAA-3′ and RP 5′-TGGG AGTTTCTGCGTTGGTG -3′) were used.

Pathogen infections and tissue staining.

H. parasitica was grown, propagated, and applied to *Arabidopsis* as previously described (McDowell et al. 2000). Two-

week-old seedlings were spray inoculated with *H. parasitica* spore suspensions (3×10^4 to 1×10^5 spores/ml of water as noted) with Preval sprayers. *H. parasitica* growth was determined 7 days postinfection by Trypan Blue staining, visual sporangiophore counts, or by using a hemacytometer to determine the spore density of a suspension of 20 mg of fresh weight of infected tissue in 10 ml of water. Trypan Blue and 2,4-diaminobenzidine staining was performed as previously described (McDowell et al. 2000; Torres et al. 2002). The Mann-Whitney U test was used to determine if the effects of the *wrky70* mutants on sporulation were statistically significant. This is a nonparametric test of statistical significance that can be used to test whether the observations in one sample tend to be larger than the observations in a second, independent sample (Zar 1999).

RNA gel blot analysis.

Total RNA was isolated from infected and uninfected seedlings using TRIZOL (Invitrogen). RNA (15 μg) was loaded on formaldehyde agarose gels with ethidium bromide for gel electrophoresis. Gels were photographed with UV light to assess loading. RNA was transferred to Hybond XL Nylon membranes (Amersham) and hybridized to PCR-amplified probes labeled with ^{32}P by random priming following the manufacturer's instructions (Stratagene). Hybridizations and washings were done using ULTAhyb hybridization buffer (Ambion) following the manufacturer's instructions. Primers used for probe amplification were *AtWRKY70* (FP 5′-CAAACCACACCAAG AGGAAAG-3′, RP 5′-CACTCATTAGAGAAAAGGGCAAA-3′), *LURP1* (FP 5′-AAAGTATGCAGCAGCCCTGTG-3′, RP 5′-GCTCCAGAACAATCAGCAAG-3′), and *CaBP22* (FP 5′-GTGCGCAATGGCTAAGGACAC-3′, RP 5′-GATTCTTGGC CATCATAAGCC-3′).

INA treatment.

Two-week-old seedlings were sprayed with 0.33 mM INA 2 days prior to *H. parasitica* spray inoculation. *H. parasitica* growth was determined 7 days after *H. parasitica* infection.

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LITERATURE CITED

Alonso, J. M., Stepanova, A. N., Leisse, T. J., Kim, C. J., Chen, H., Shinn, P., Stevenson, D. K., Zimmerman, J., Barajas, P., Cheuk, R., Gadrinab, C., Heller, C., Jeske, A., Koesema, E., Meyers, C. C., Parker, H., Prednis, L., Ansari, Y., Choy, N., Deen, H., Geralt, M., Hazari, N., Hom, E., Karnes, M., Mulholland, C., Ndubaku, R., Schmidt, I., Guzman, P., Aguilar-Henonin, L., Schmid, M., Weigel, D., Carter, D. E., Marchand, T., Risseeuw, E., Brogden, D., Zeko, A., Crosby, W. L., Berry, C. C., and Ecker, J. R. 2003. Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. Science 301:653-657.

Asai, T., Tena, G., Plotnikova, J., Willmann, M. R., Chiu, W.-L., Gomez-Gomez, L., Boller, T., Ausubel, F. M., and Sheen, J. 2002. MAP kinase signaling cascade in *Arabidopsis* innate immunity. Nature 415:977-980.

Bartsch, M., Gobbato, E., Bednarek, P., Debey, S., Schultze, J. L., Bautor, J., and Parker, J. E. 2006. Salicylic acid-independent ENHANCED DISEASE SUSCEPTIBILITY1 signaling in *Arabidopsis* immunity and cell death is regulated by the monooxygenase FMO1 and the Nudix hydrolase NUDT7. Plant Cell 18:1038-1051.

Cao, H., Bowling, S. A., Gordon, S., and Dong, X. 1994. Characterization of an *Arabidopsis* mutant that is non-responsive to inducers of systemic acquired resistance. Plant Cell 6:1583-1592.

Cao, H., Glazebrook, J., Clark, J. D., Volko, S., and Dong, X. 1997. The *Arabidopsis NPR1* gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. Cell 88:57-64.

Chen, C., and Chen, Z. 2002. Potentiation of developmentally regulated plant defense response by AtWRKY18, a pathogen-induced *Arabidopsis* transcription factor. Plant Physiol. 129:706-716.

Chen, W., Provart, N. J., Glazebrook, J., Katagiri, F., Chang, H. S., Eulgem, T., Mauch, F., Luan, S., Zou, G., Whitham, S. A., Budworth, P. R., Tao, Y., Xie, Z., Chen, X., Lam, S., Kreps, J. A., Harper, J. F., Si-Ammour, A., Mauch-Mani, B., Heinlein, M., Kobayashi, K., Hohn, T., Dangl, J. L., Wang, X., and Zhu, T. 2002. Expression profile matrix of *Arabidopsis* transcription factor genes suggests their putative functions in response to environmental stresses. Plant Cell 14:559-574.

Chisholm, S. T., Coaker, G., Day, B., and Staskawicz, B. J. 2006. Host-microbe interactions: Shaping the evolution of the plant immune response. Cell 124:803-814.

Dangl, J. L., and Jones, J. D. G. 2001. Plant pathogens and integrated defence responses to infection. Nature 411:826-833.

Delaney, T., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gut-Rella, M., Kessman, H., Ward, E., and Ryals, J. 1994. A central role of salicylic acid in plant disease resistance. Science 266:1247-1250.

Delledonne, M., Murgia, I., D. E., Sbicego, P. F., Biondani, A., Polverari, A., and Lamb, C. 2002. Reactive Oxygen intermediates modulate nitric oxide signaling in the plant hypersensitive disease-resistance response. Plant. Physiol. Biochem. 40:605-610.

Deslandes, L., Olivier, J., Peeters, N., Feng, D. X., Khounlotham, M., Boucher, C., Somssich, I., Genin, S., and Marco, Y. 2003. Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. Proc. Natl. Acad. Sci. U.S.A. 100:8024-8029.

Deslandes, L., Olivier, J., Theulieres, F., Hirsch, J., Feng, D. X., Bittner-Eddy, P., Beynon, J., and Marco, Y. 2002. Resistance to *Ralstonia solanacearum* in *Arabidopsis thaliana* is conferred by the recessive *RRS1-R* gene, a member of a novel family of resistance genes. Proc. Natl. Acad. Sci. U.S.A. 99:2404-2409.

Desveaux, D., Subramaniam, R., Despres, C., Mess, J. N., Levesque, C., Fobert, P. R., Dangl, J. L., and Brisson, N. 2004. A "Whirly" transcription factor is required for salicylic acid-dependent disease resistance in *Arabidopsis*. Dev. Cell 6:229-240.

Dong, J., Chen, C., and Chen, Z. 2003. Expression profiles of the Arabidopsis WRKY gene superfamily during plant defense response. Plant Mol. Biol. 51:21-37.

Dong, X. 2004. NPR1, all things considered. Curr. Opin. Plant Biol. 7:547-552.

Durner, J., and Klessig, D. F. 1999. Nitric oxide as a signal in plants. Curr. Opin. Plant Biol. 2:369-374.

Eulgem, T. 2005. Regulation of the *Arabidopsis* defense transcriptome. Trends Plant Sci. 10:71-78.

Eulgem, T., Rushton, P. J., Robatzek, S., and Somssich, I. E. 2000. The WRKY superfamily of plant transcription factors. Trends Plant Sci. 5:199-206.

Eulgem, T., Rushton, P. J., Schmelzer, E., Hahlbrock, K., and Somssich, I. E. 1999. Early nuclear events in plant defence signaling: Rapid activation by WRKY transcription factors. EMBO (Eur. Mol. Biol. Organ.) J. 18.

Eulgem, T., Weigman, V. J., Chang, H.-S., McDowell, J. M., Holub, E. B., Glazebrook, J., Zhu, T. A., and Dangl, J. L. 2004. Gene expression signatures from three genetically separable resistance gene signaling pathways for downy mildew resistance. Plant Physiol. 135:1129–1144.

Flor, H. H. 1971. Current status of the gene-for-gene concept. Annu. Rev. Phytopathol. 9:275-296.

Glazebrook, J. 2001. Genes controlling expression of defense responses in *Arabidopsis*—2001 status. Curr. Opin. Plant Biol. 4:301-308.

Glazebrook, J., Chen, W., Estes, B., Chang, H. S., Nawrath, C., Metraux, J. P., Zhu, T., and Katagiri, F. 2003. Topology of the network integrating salicylate and jasmonate signal transduction derived from global expression phenotyping. Plant J. 34:217-228.

Glazebrook, J., Rogers, E. E., and Ausubel, F. M. 1996. Isolation of *Arabidopsis* mutants with enhanced disease susceptibility by direct screening. Genetics 143:973-982.

Glazebrook, J., Zook, M., Mert, F., Kagan, I., Rogers, E. E., Crute, I. R., Holub, E. B., and Ausubel, F. M. 1997. Phytoalexin-deficient mutants of *Arabidopsis* reveal that *PAD4* encodes a regulatory factor and that four *PAD* genes contribute to downy mildew resistance. Genetics 146:381-392.

Hammond-Kosack, K. E., and Parker, J. E. 2003. Deciphering plant-pathogen communication: Fresh perspectives for molecular resistance breeding. Curr. Opin. Biotechnol. 14:177-193.
Holub, E., Brose, E., Tör, M., Clay, C., Crute, I. R., and Beynon, J. L. 1995. Phenotypic and genotypic variation in the interaction between *Arabidopsis thaliana* and *Albugo candida.* Mol. Plant-Microbe Interact. 8:916-928.
Holub, E. B., Beynon, J. L., and Crute, I. R. 1994. Phenotypic and genotypic characterization of interactions between isolates of *Peronospora parasitica* and accessions of *Arabidopsis thaliana.* Mol. Plant-Microbe Interact. 7:223-239.
Jabs, T., Colling, C., Tschöpe, M., Hahlbrock, K., and Scheel, D. 1997. Elicitor-stimulated ion fluxes and reactive oxygen species from the oxidative burst signal defense gene activation and phytoalexin synthesis in parsley. Proc. Natl. Acad. Sci. U.S.A. 94:4800-4805.
Jirage, D., Tootle, T. L., Reuber, T. L., Frost, L. N., Feys, B. J., Parker, J. E., Ausubel, F. M., and Glazebrook, J. 1999. *Arabidopsis thaliana PAD4* encodes a lipase-like gene that is important for salicylic acid signaling. Proc. Natl. Acad. Sci. U.S.A. 96:135883-113588.
Kalde, M., Barth, M., Somssich, I. E., and Lippok, B. 2003. Members of the *Arabidopsis* WRKY group III transcription factors are part of different plant defense signaling pathways. Mol Plant-Microbe Interact. 16:295-305.
Katagiri, F. 2004. A global view of defense gene expression regulation—A highly interconnected signaling network. Curr. Opin. Plant Biol. 7:506-511.
Kessmann, H., Staub, T., Hofmann, C., Ahl Goy, P., Ward, E., Uknes, S., and Ryals, J. 1993. Induced disease resistance by isonicotinic acid derivatives. Jpn. J. Pestic. Sci. 10:29-37.
Kessmann, H., Staub, T., Hofmann, C., Maetzke, T., Herzog, J., Ward, E., Uknes, S., and Ryals, J. 1994. Induction of systemic acquired resistance in plants by chemicals. Annu. Rev. Phytopathol 32:439-459.
Kinkema, M., Fan, W., and Dong, X. 2000. Nuclear localization of NRP1 is required for activation of *PR* gene expression. Plant Cell 12:2339-2350.
Klessig, D. F., Durner, J., Noad, R., Navarre, D. A., Wendehenne, D., Kumar, D., Zhou, J. M., Shah, J., Zhang, S., Kachroo, P., Trifa, Y., Pontier, D., Lam, E., and Silva, H. 2000. Nitric oxide and salicylic acid signaling in plant defense. Proc. Natl. Acad. Sci. U.S.A. 97:8849-8855.
Kunkel, B. N., and Brooks, D. M. 2002. Cross talk between signaling pathways in pathogen defense. Curr. Opin. Plant. Biol. 5:325-331.
Lamb, C., and Dixon, R. A. 1997. The oxidative burst in plant disease resistance. Annu. Rev. Physiol. Plant Mol. Biol. 48:251-275.
Levine, A., Tenhaken, R., Dixon, R., and Lamb, C. J. 1994. H2O2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell 79:583-593.
Li, J., Brader, G., Kariola, T., and Palva, E. T. 2006. WRKY70 modulates the selection of signaling pathways in plant defense. Plant J. 46:477-491.
Li, J., Brader, G., and Palva, E. T. 2004. The WRKY70 transcription factor: A node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. Plant Cell 16:319-331.
Liu, Y., Schiff, M., and Dinesh-Kumar, S. P. 2004. Involvement of MEK1 MAPKK, NTF6 MAPK, WRKY/MYB transcription factors, COI1 and CTR1 in N-mediated resistance to *Tobacco mosaic virus*. Plant J. 38:800-809.
Maeo, K., Hayashi, S., Kojima-Suzuki, H., Morikami, A., and Nakamura, K. 2001. Role of conserved residues of the WRKY domain in the DNA-binding of tobacco WRKY family proteins. Biosci. Biotechnol. Biochem. 65:2428-2436.
Maleck, K., Levine, A., Eulgem, T., Morgan, A., Schmid, J., Lawton, K. A., Dangl, J. L., and Dietrich, R. A. 2000. The transcriptome of Arabidopsis thaliana during systemic acquired resistance. Nat. Genet. 26:403-410.
McDowell, J. M., Cuzick, A., Can, C., Beynon, J., Dangl, J. L., and Holub, E. B. 2000. Downy mildew (*Peronospora parasitica*) resistance genes in *Arabidopsis* vary in functional requirements for *NDR1*, *EDS1*, *NPR1*, and salicylic acid accumulation. Plant J. 22:523-530.
Mou, Z., Fan, W., and Dong, X. 2003. Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. Cell 113:935-944.
Navarro, L., Zipfel, C., Rowland, O., Keller, I., Robatzek, S., Boller, T., and Jones, J. D. 2004. The transcriptional innate immune response to flg22. Interplay and overlap with *Avr* gene-dependent defense responses and bacterial pathogenesis. Plant Physiol. 135:1113-1128.
Nawrath, C., Heck, S., Parinthawong, N., and Metraux, J.-P. 2002. EDS5, an essential component of salicylic acid-dependent signaling for disease resistance in *Arabidopsis*, is a member of the MATE transporter family. Plant Cell 14:275-286.
Nurnberger, T., Brunner, F., Kemmerling, B., and Piater, L. 2004. Innate immunity in plants and animals: Striking similarities and obvious differences. Immunol. Rev. 198:249-266.
Petersen, M., Brodersen, P., Naested, H., Andreasson, E., Lindhart, U., Johanson, B., Nielsen, H. B., Lacy, M., Austin, M. J., Parker, J. E., Sharma, S. B., Klessig, D. F., Martienssen, R., Mattsson, O., Jensen, A. B., and Mundy, J. 2000. *Arabidopsis* map kinase 4 negatively regulates systemic acquired resistance. Cell 103:1111-1120.
Piedras, P., Hammond-Kosack, K. E., Harrison, K., and Jones, J. D. G. 1998. Rapid, Cf-9 and Avr9 dependent, production of active oxygen species in tobacco suspension cultures. Mol. Plant-Microbe Interact. 11:1155-1166.
Ramonell, K., Berrocal-Lobo, M., Koh, S., Wan, J., Edwards, H., Stacey, G., and Somerville, S. 2005. Loss-of-function mutations in chitin responsive genes show increased susceptibility to the powdery mildew pathogen *Erysiphe cichoracearum*. Plant Physiol. 138:1027-1036.
Ramonell, K., Zhang, B., Ewing, R. M., Chen, Y., Xu, D., Stacey, G., and Somerville, S. 2002. Microarray analysis of chitin elicitation in *Arabidopsis thaliana*. Mol. Plant Pathol. 3:301-311.
Rowland, O., Ludwig, A. A., Merrick, C. J., Baillieul, F., Tracy, F. E., Durrant, W. E., Fritz-Laylin, L., Nekrasov, V., Sjolander, K., Yoshioka, H., and Jones, J. D. 2005. Functional analysis of Avr9/Cf-9 rapidly elicited genes identifies a protein kinase, ACIK1, that is essential for full Cf-9-dependent disease resistance in tomato. Plant Cell 17:295-310.
Rushton, P. J., Tovar Torres, J., Parniske, M., Wernert, P., Hahlbrock, K., and Somssich, I. E. 1996. Interaction of elicitor-induced DNA-binding proteins with elicitor response elements in the promoters of parsley *PR1* genes. EMBO (Eur. Mol. Biol. Organ.) J. 15:5690-5700.
Ryals, J., Weymann, K., Lawton, K., Friedrich, L., Ellis, D., Steiner, H. Y., Johnson, J., Delaney, T. P., Jesse, T., Vos, P., and Uknes, S. 1997. The *Arabidopsis* NIM1 protein shows homology to the mammalian transcription factor inhibitor I kappa B. Plant Cell 9:425-439.
Schenk, P. M., Kazan, K., Wilson, I., Anderson, J. P., Richmond, T., Somerville, S. C., and Manners, J. M. 2000. Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. Proc. Natl. Acad. Sci. U.S.A. 97:11655-11660.
Sessions, A., Burke, E., Presting, G., Aux, G., McElver, J., Patton, D., Dietrich, B., Ho, P., Bacwaden, J., Ko, C., Clarke, J. D., Cotton, D., Bullis, D., Snell, J., Miguel, T., Hutchison, D., Kimmerly, B., Mitzel, T., Katagiri, F., Glazebrook, J., Law, M., and Goff, S. A. 2002. A high-throughput *Arabidopsis* reverse genetics system. Plant Cell 14:2985-2994.
Shapiro, A. D., and Zhang, C. 2001. The role of NDR1 in avirulence gene-directed signaling and control of programmed cell death in *Arabidopsis*. Plant Physiol. 127:1089-1101.
Slusarenko, A. J., and Schlaich, N. L. 2003. Downy mildew of *Arabidopsis thaliana* caused by *Hyaloperonospora parasitica* (formerly *Peronospora parasitica*). Mol. Plant Pathol. 4:159-170.
Somssich, I., and Hahbrock, K. 1998. Pathogen defence in plants—A paradigm of biological complexity. Trends Plant Sci. 3:86-90.
Spoel, S. H., Koornneef, A., Claessens, S. M., Korzelius, J. P., Van Pelt, J. A., Mueller, M. J., Buchala, A. J., Metraux, J. P., Brown, R., Kazan, K., Van Loon, L. C., Dong, X., and Pieterse, C. M. 2003. NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. Plant Cell 15:760-770.
Sun, C., Palmqvist, S., Olsson, H., Boren, M., Ahlandsberg, S., and Jansson, C. 2003. A novel WRKY transcription factor, SUSIBA2, participates in sugar signaling in barley by binding to the sugar-responsive elements of the iso1 promoter. Plant Cell 15:2076-2092.
Tao, Y., Xie, Z., Chen, W., Glazebrook, J., Chang, H. S., Han, B., Zhu, T., Zou, G., and Katagiri, F. 2003. Quantitative nature of *Arabidopsis* responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. Plant Cell 15:317-330.
Torres, M. A., and Dangl, J. L. 2005. Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. Curr. Opin. Plant Biol. 8:397-403.
Torres, M. A., Dangl, J. L., and Jones, J. D. G. 2002. *Arabidopsis* gp91-phox homologues *AtrbohD* and *AtrbohF* are required for accumulation of reactive oxygen intermediates in the plant defense response. Proc. Natl. Acad. Sci. U.S.A. 99:523-528.
Turck, F., Zhou, A., and Somssich, I. E. 2004. Stimulus-dependent, promoter-specific binding of transcription factor WRKY1 to its native promoter and the defense-related gene *PcPR1-1* in parsley. Plant Cell 16:2573-2585.
Uknes, S., Mauch-Mani, B., Moyer, M., Potter, S., Williams, S., Dincher, S., Chandler, D., Slusarenko, A., Ward, E., and Ryals, J. 1992. Acquired resistance in *Arabidopsis*. Plant Cell 4:645-656.
Ulker, B., and Somssich, I. E. 2004. WRKY transcription factors: From DNA binding towards biological function. Curr. Opin. Plant Biol. 7:491-498.
van der Biezen, E. A., Freddie, C. T., Kahn, K., Parker, J. E., and Jones, J. D. G. 2002. *Arabidopsis RPP4* is a member the *RPP5* multigene family

of TIR-NB-LRR genes and confers downy mildew resistance through multiple signaling components. Plant J. 29:439-451.
Veronese, P., Nakagami, H., Bluhm, B., Abuqamar, S., Chen, X., Salmeron, J., Dietrich, R. A., Hirt, H., and Mengiste, T. 2006. The membrane-anchored BOTRYTIS-INDUCED KINASE1 plays distinct roles in Arabidopsis resistance to necrotrophic and biotrophic pathogens. Plant Cell 18:257-273.
White, R. F. 1979. Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. Virology 99:410-412.
Wildermuth, M. C., Dewdney, J., Wu, G., and Ausubel, F. M. 2001. Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature 414:562-565.
Wu, K. L., Guo, Z. J., Wang, H. H., and Li, J. 2005. The WRKY family of transcription factors in rice and *Arabidopsis* and their origins. DNA Res. 12:9-26.
Xu, X., Chen, C., Fan, B., and Chen, Z. 2006. Physical and functional interactions between pathogen-induced *Arabidopsis* WRKY18, WRKY40, and WRKY60 transcription factors. Plant Cell 18:1310-1326.
Yu, D., Chen, C., and Chen, Z. 2001. Evidence for an important role of WRKY DNA binding proteins in the regulation of NPR1 gene expression. Plant Cell 13:1527-1540.
Zar, J., H 1999. Biostatistical Analysis, 4th ed. Prentice Hall, Upper Saddle River, NJ, U.S.A.
Zimmermann, P., Hirsch-Hoffmann, M., Hennig, L., and Gruissem, W. 2004. GENEVESTIGATOR. *Arabidopsis* microarray database and analysis toolbox. Plant Physiol. 136:2621-2632.

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