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Regulation of plant innate immunity by three proteins in a complex conserved across the plant and animal kingdoms

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Innate immunity against pathogen infection is an evolutionarily conserved process among multicellular organisms. *Arabidopsis SNC1* encodes a Resistance protein that combines attributes of multiple mammalian pattern recognition receptors. Utilizing *snc1* as an autoimmune model, we identified a discrete protein complex containing at least three members—MOS4 (Modifier Of *snc1*, 4), AtCDC5, and PRL1 (Pleiotropic Regulatory Locus 1)—that are all essential for plant innate immunity. AtCDC5 has DNA-binding activity, suggesting that this complex probably regulates defense responses through transcriptional control. Since the complex components along with their interactions are highly conserved from fission yeast to *Arabidopsis* and human, they may also have a yet-to-be-identified function in mammalian innate immunity.

[*Keywords*: Innate immunity; *Arabidopsis*; MOS4; PRL1; AtCDC5; NTC; MAC]

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Innate immunity is the front-line defense system of multicellular eukaryotes and is activated by a limited number of pattern recognition receptors (PRRs). Most PRRs in animals contain leucine-rich repeat (LRR) motifs including Toll-like receptors (TLRs) and proteins with a nucleotide oligomerization domain (NOD) (Akira et al. 2006). There is considerable evidence that plant and animal innate immune systems are conserved as a consequence of convergent evolution (Ausubel 2005). Initiation of induced plant defense signaling involves the recognition of specific pathogen effectors by the products of specialized host *RESISTANCE* (*R*) genes (Belkhadir et al. 2004). TIR-NB-LRR-type R proteins combine features of two different classes of PRRs in animal innate immunity: TLRs and NOD proteins (Philpott and Girardin 2004). In the unique *Arabidopsis snc1* mutant, a gain-of-function mutation in a TIR-NB-LRR-type *R* gene results in constitutive defense responses against a wide spectrum of pathogens—the specific pathogen effector detected by wild-type SNC1 in nature is unknown. The *snc1* mutation is located in the linker region between the NB and LRR (Zhang et al. 2003). Intriguingly, mutations in NODs are associated with autoimmune disorders such as Crohn's disease and Blau syndrome, and mutations in the corresponding linker region of some mammalian NODs result in constitutive self-activation of innate immune responses (Eckmann and Karin 2005).

To identify additional components required for plant innate immunity, a suppressor screen was performed to search for mutations that revert the autoimmune phenotypes of *snc1* to wild type. One mutant, *modifier of snc1, 4* (*mos4*), completely abolished enhanced resistance to the virulent pathogens in *snc1*. *MOS4* encodes a nuclear protein homologous to human Breast Cancer-Amplified Sequence 2 (BCAS2). MOS4 directly interacts in planta with AtCDC5, an atypical R2R3 Myb transcription factor with significant homology with human CDC5L, which is a key component of the spliceosome-associated PRP Nineteen Complex (NTC). BCAS2 also belongs to NTC. AtCDC5 was previously shown to have sequence-specific DNA-binding activity (Hirayama and Shinozaki 1996); thus it most likely functions as a transcriptional regulator. A third member of the NTC that interacts directly with CDC5L is PLRG1 (Ajuh et al. 2001). In *Arabidopsis*, one of the PLRG1 homologs, Pleiotropic Regulatory Locus 1 (PRL1), interacted with AtCDC5 in planta. MOS4, AtCDC5, and PRL1 are all required for pathogen resistance in *Arabidopsis*. Thus, the evolutionarily conserved NTC plays an essential regulatory role in plant innate immunity and may have a similar function in animals.

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Results

mos4-1 *suppresses the autoimmunity-related phenotypes of* snc1

In plants, innate immunity against microbial pathogens is governed by R proteins that are responsible for recognizing pathogen effectors and initiating downstream defense responses (Belkhadir et al. 2004). The most common R proteins are TIR- and CC-class NB-LRR proteins (Chisholm et al. 2006). Although many *R* genes have been cloned, the signal transduction events downstream from R proteins remain scarcely detailed. In order to identify more signaling components downstream from R-protein activation, we carried out a suppressor screen using *snc1* (Zhang and Li 2005; Y. Zhang et al. 2005). The *mos4-1 snc1* double-mutant plants were the same size as wild type, and had leaves that were less curly than in *snc1* (Fig. 1A). Constitutive *pBGL2-GUS* reporter gene expression in *snc1* was completely abolished by *mos4-1* (Supplementary Fig. 1A). Furthermore, RT–PCR analysis showed that endogenous *PR-2* (*BGL2*) was no longer constitutively expressed in *mos4-1 snc1* plants, and that the expression of *PR-1* was partially suppressed (Supplementary Fig. 1B). Genetic analysis indicated that *mos4-1* is a single recessive mutation (data not shown). In addition, defects in MOS4 affected flowering time and male fertility (Supplementary Fig. 2).

The *snc1* mutant accumulates high levels of the defense-signaling molecule salicylic acid (SA). Previously, we found that blocking SA synthesis only partially suppressed *snc1* phenotypes (Zhang et al. 2003). In *mos4-1 snc1*, levels of free SA were about eightfold lower than in *snc1*, and levels of total SA were ~30-fold lower than in *snc1* (Fig. 1B); these SA levels were comparable with wild-type controls, suggesting that MOS4 functions upstream of SA synthesis.

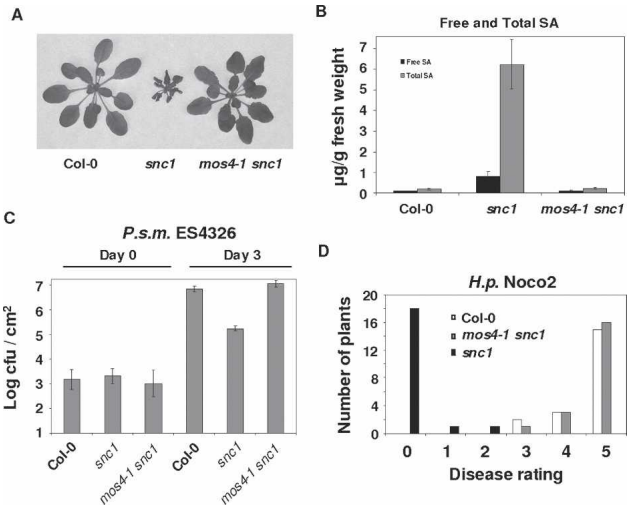

Figure 1. Suppression of *snc1*-associated morphology, SA accumulation and pathogen resistance by *mos4-1*. (*A*) Morphology of wild-type Columbia (Col-0), *snc1*, and *mos4-1 snc1*. Image is of representative plants. (*B*) Free and total SA in leaves of Col-0, *snc1*, and *mos4-1 snc1* plants. (*C*) Growth of *P.s.m.* ES4326 at 0 and 3 DPI with OD_{600} = 0.001. The log-transformed values presented are averages of four replicates ± SD. (*D*) Growth of *H.p.* Noco2 (see the Supplemental Material). Data are presented for 20 plants of each genotype. The experiments were repeated at least twice with similar results.

Two of the most amenable plant–pathogen model systems for the genetic dissection of host responses are those between *Arabidopsis* and (1) *Pseudomonas syringae* pv *maculicola* (*P.s.m.*) and pv tomato (*P.s.t.*), two strains of a hemi-biotrophic bacterial species that cause disease symptoms ranging from leaf spots to stem cankers, on a broad range of plant hosts; and (2) *Hyaloperonospora parasitica* (*H.p.*), an obligate biotrophic oomycete. Oomycetes grow funguslike hyphae but have cellulosic cell walls and are phylogenetically unrelated to fungi. *snc1* is resistant to *P.s.m.* ES4326 and *H.p.* Noco2, both of which are normally virulent in *Arabidopsis* Col-0 plants. While *snc1* had no disease symptoms and supported ~50 times less bacterial growth than wild type at 3 d post-infection (DPI), *mos4-1 snc1* harbored an approximately wild-type titer of bacterial growth (Fig. 1C) and exhibited some disease symptoms (data not shown). Furthermore, *mos4-1* completely suppressed resistance to *H.p.* Noco2 in *snc1*, as shown in Figure 1D.

MOS4 *encodes the* Arabidopsis *homolog of human BCAS2*

MOS4 (At3g18165) was cloned using a map-based approach (see the Supplemental Material; Supplementary Fig. 3). The mutation in *mos4-1 snc1* was found to be an insertion in *At3g18165*, and *35S-At3g18165* was able to complement the *mos4*-related phenotypes (Supplementary Fig. 3E,F). In addition, other T-DNA alleles of *At3g18165* were able to suppress *snc1* (Supplementary Fig, 3G), confirming that *MOS4* is indeed *At3g18165*. Sequence analysis revealed that MOS4 is similar to human *BCAS2*, with 29% identity and 48% similarity at the protein level. An amino acid alignment between *Arabidopsis* MOS4 and related proteins from other eukaryotes shows highly conserved regions (Supplementary Fig. 4). Since the identity of MOS4 did not reveal the detailed biochemical mechanism of its regulation of innate immunity, we employed three approaches to further elucidate MOS4's detailed modes of action: (1) genetic analysis to identify the pathway in which MOS4 acts, (2) subcellular localization of MOS4, and (3) identification of MOS4-interacting proteins.

MOS4 is essential for signaling in the NPR1-independent pathway

To understand how MOS4 contributes to plant innate immunity, we used a genetic approach to examine the pathway in which it functions. Signaling downstream from *snc1* diverges into multiple pathways dependent on different signaling intermediates (Zhang et al. 2003). NPR1, an ankyrin-repeat protein with redox-regulated nuclear translocation, controls basal resistance down-

stream from SA and regulates *PR-1* expression through interaction with the TGA subfamily of bZIP transcription factors (Dong 2004). Since *npr1-1* does not block *PR-2* expression or resistance to virulent *P.s.m.* ES4326 in the *snc1 npr1-1* double mutant, *snc1* activates NPR1-dependent and independent responses (Zhang et al. 2003). Because *mos4-1* completely suppressed the constitutive *PR-2* expression of *snc1* (Supplementary Fig. 1B), we surmised that MOS4 might be required for the NPR1-independent pathway. To test the epistatic relationship between *MOS4* and *NPR1*, *mos4-1 npr1-1* double and *mos4-1 npr1-1 snc1* triple mutants were created. When challenged with virulent and avirulent pathogens, *mos4-1* completely suppressed the enhanced resistance and morphology of *snc1 npr1* in the *mos4-1 npr1-1 snc1* triple mutant, and supported at least a 10-fold higher titer of bacteria than *mos4-1 snc1*, suggesting that a defect in MOS4 blocks NPR1-independent pathways downstream from *snc1* (Fig. 2A). Furthermore, in the absence of the *snc1* mutation, *mos4-1 npr1* plants were more susceptible to a normally subclinical concentration of *P.s.m.* ES4326 than *npr1-1* or *mos4-1* alone, supporting three- to sevenfold more bacteria 3 DPI (Fig. 2B). The additive nature of the enhanced disease susceptibility (EDS) of *mos4-1* and *npr1-1* shown by these data suggests that two separate pathways contributing to basal defense are compromised in the double mutant. In addition, *mos4-1 npr1-1* was more susceptible to avirulent *H.p.* Emwa1 than either the *npr1-1* or *mos4-1* single mutants (Fig. 2C), indicating that NPR1-independent signaling downstream from R protein activation—in this case, RPP4—is mediated by MOS4.

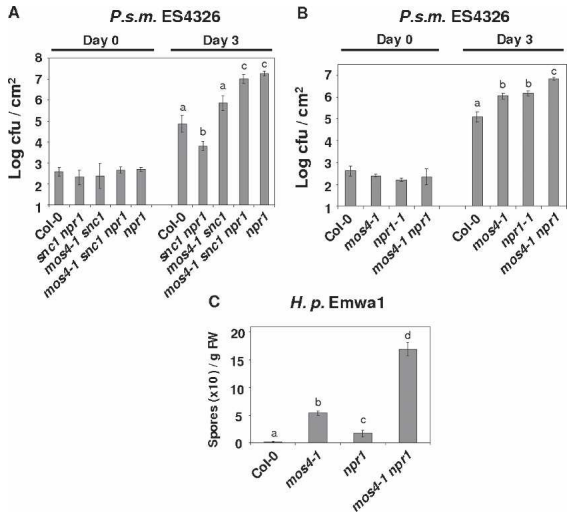

Figure 2. *mos4-1* affects the NPR1-independent signaling pathway. (*A*) Growth of *P.s.m.* ES4326 in Col-0, *snc1 npr1-1*, *mos4-1 snc1*, *mos4-1 snc1 npr1-1*, and *npr1-1* after infiltration with OD_{600} = 0.0001. (*B*) Growth of *P.s.m.* ES4326 in Col-0, *mos4-1*, *npr1-1*, and *mos4-1 npr1-1* after infiltration with OD_{600} = 0.00005. The log-transformed values presented are averages of four replicates ± SD. (*C*) Growth of avirulent *H.p.* Emwa1 on Col-0, *mos4-1*, *npr1-1*, and *mos4-1 npr1-1* plants. Results are averages of six replicates ± SD. The log-transformed values presented are averages of four replicates ± SD. The experiments were repeated at least twice with similar results. (a–d) Statistically significant ($P < 0.0001$) difference as determined by unpaired Student's *t*-test.

To date, a fairly exclusive group of NPR1-independent defense signaling components has been described; these include MOS2, a putative RNA-binding protein isolated earlier from the *snc1* suppressor screen (Y. Zhang et al. 2005), and FMO1 (FLAVIN-DEPENDENT MONOXYGENASE 1), a positive regulator of EDS1 signaling that is independent of SA accumulation (Bartsch et al. 2006). Although the genetic relationship of *FMO* with *NPR1* remains to be determined, it is likely to be NPR1 independent.

Because the *mos4-1* single mutant is able to accumulate SA in response to avirulent pathogen infiltration (Supplementary Fig. 5), MOS4 does not seem to contribute to SA accumulation caused by pathogen infection. The reason why *mos4-1 snc1* has low SA is probably due to the SA-independent pathway having a positive amplification role in SA synthesis (Y. Zhang et al. 2005). A schematic model of MOS4 signaling in plant innate immunity is shown in Supplementary Figure 6.

MOS4 is a nuclear protein

To determine the subcellular localization of MOS4, GFP was cloned in-frame at the C terminus of MOS4 and transformed into *mos4-1* plants. GFP fluorescence was detected exclusively in the nucleus of various cell types in several independent *MOS4-GFP* transgenic lines, including guard cells (Fig. 3A), epidermal cells, and root cells (data not shown), suggesting that MOS4 is a nuclear protein. When *MOS4-GFP* was transformed into *mos4-1 snc1* plants and the transgenic progeny examined, all of the nine transgenic plants selected exhibited *snc1* phenotypes, indicating that the expression of *MOS4-GFP* driven by its native promoter complements the *mos4-1* mutation in the *snc1* background (Fig. 3B). In addition, MOS4-GFP complemented the EDS phenotype of the *mos4-1* single mutant (Fig. 3C). Although *mos4-1* with *MOS4-GFP* is wild-type-like, whereas *mos4-1 snc1* with *MOS4-GFP* is *snc1*-like, we did not observe any difference in GFP localization or intensity of GFP fluorescence between the two genetic backgrounds (data not shown).

MOS4 interacts in yeast with AtCDC5, an atypical R2R3 Myb transcription factor homologous to human CDC5L

Analysis of the MOS4 amino acid sequence predicted that the C-terminal half had the potential to form an amphipathic α-helix, a motif known to mediate protein–protein interactions through parallel two-stranded coiled-coil structure. We thus employed a GAL4-based yeast two-hybrid screen to search for proteins that are capable of interacting with MOS4. One of the interacting proteins found in the screen was AtCDC5 (Supplemen-

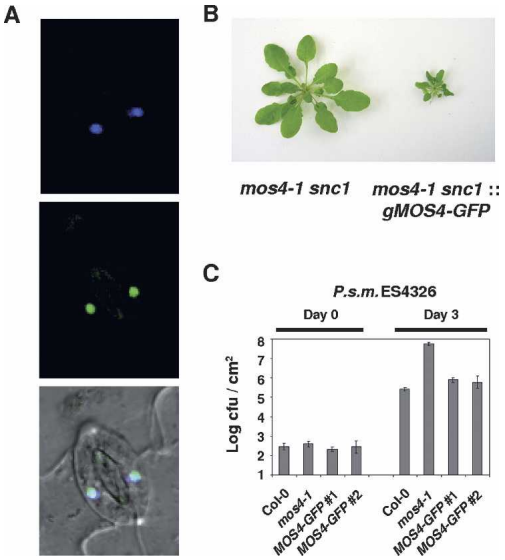

Figure 3. Subcellular localization of MOS4. (*A*) Guard cells of transgenic *mos4-1* plants expressing the genomic *MOS4:GFP* fusion protein were examined by confocal microscopy; a representative plant is shown. (*Top* panel) DAPI staining of the nucleus. (*Middle* panel) MOS4-GFP fluorescence. (*Bottom* panel) Merged fluorescence channels and bright-field image. (*B*) Morphology of *mos4-1 snc1* complemented with *gMOS4-GFP*. (*C*) Growth of *P.s.m.* ES4326 in Col-0, *mos4-1*, and two independent complementing lines of *gMOS4-GFP* transformed into *mos4-1* mutants at 0 and 3 DPI, showing complementation of EDS in *mos4-1* gMOS4-GFP. A subclinical concentration of *P.s.m.* (OD_{600} = 0.0001) was infiltrated. The log-transformed values presented are averages of four replicates ± SD. The experiment was repeated twice with similar results.

tary Fig. 7A), an 844-amino-acid polypeptide with similarity to R2R3-type MYB transcription factors that exhibits in vitro sequence-specific DNA-binding activity (Hirayama and Shinozaki 1996). To confirm in planta interaction of MOS4 and AtCDC5, HA or GFP was cloned in-frame at the C ternimus of MOS4 driven by its own promoter and transformed into *mos4-1*. The fusion clone is able to fully complement *mos4*-related phenotypes (data not shown), indicating that the fusion proteins function the same as the wild type. Immunoprecipitation (IP) using Anti-GFP Microbeads followed by Western blot analysis with AtCDC5 antibody showed that AtCDC5 coimmunoprecipitated with MOS4-GFP (Fig. 4A). Coimmunoprecipitation (co-IP) with Anti-HA Microbeads yielded similar results (data not shown).

The human homologs of MOS4 and AtCDC5, BCAS2 (also known as hSPF27) and hCDC5L, respectively, have been isolated as components of the multiprotein spliceosome complex by proteomic analysis (Neubauer et al. 1998; Zhou et al. 2002). Using different purification strategies and mass spectrometry, hCDC5L copurified with at least five non-small nuclear RNA (snRNA) proteins in a discrete complex, one of which was BCAS2, the MOS4 homolog (Ajuh et al. 2000). CDC5L orthologs in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*, Cdc5p and Cef1p, respectively, also copurified with a core complex nearly identical in composition to that in human cells (Ohi and Gould 2002). The complex containing Cef1p was termed the PRP NTC, based on the identity of another complex member, Prp19p (Cwf8p in *S. pombe*) (Tsai et al. 1999). The hNTC has also been termed the NMP200 Complex and PSO4 Complex (Ohi and Gould 2002). In vitro immunodepletion of the orthologous human NTC from HeLa nuclear extracts inhibits formation of pre-mRNA splicing products (Ajuh et al. 2000). Taken together, the conserved interaction between MOS4 and AtCDC5 homologs in yeast, *Arabidopsis*, and human suggests an evolutionarily conserved function of MOS4 through its interaction with AtCDC5.

AtCDC5 interacts with PRL1

A third component of the human NTC is PLRG1, a WD40 repeat protein shown to bind directly to CDC5L (Ajuh et al. 2001). An *Arabidopsis* homolog of PLRG1, the previously described *PRL1*(At4g15900), encodes a conserved nuclear WD protein that functions as a pleiotropic regulator of glucose and hormone responses (Nemeth et al. 1998). Using a yeast two-hybrid assay, we showed that AtCDC5 interacted directly with PRL1 (Supplementary Fig. 7B). In addition, PRL1-GFP was localized to the nucleus (Supplementary Fig. 8). To test for in planta interaction, GFP or HA tag was cloned in-frame at the C terminus of PRL1 and transformed into *prl1-1*. The fusion clone fully complemented all the *prl1* phe-

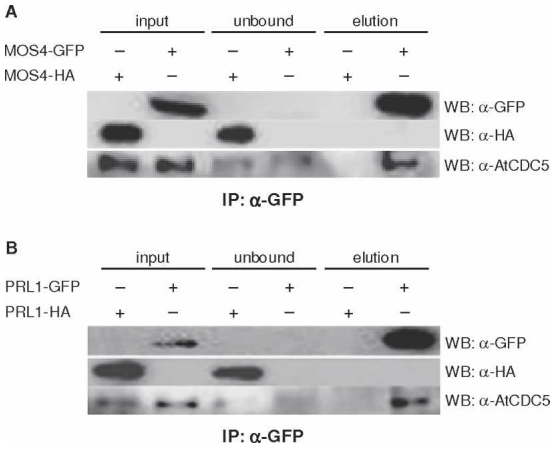

Figure 4. MOS4, AtCDC5, and PRL1 interact in planta. (*A*) AtCDC5 coimmunoprecipitates with MOS4-GFP in nuclear extracts from *mos4-1* complemented by *gMOS4-GFP* transgenic plants. (*B*) AtCDC5 coimmunoprecipitates with PRL1-GFP in nuclear extracts from *prl1-1* complemented by *gPRL1-GFP* transgenic plants. The aerial plant tissue was harvested for nuclear fractionation, and nuclear extracts were subjected to IP as indicated. Equal amounts by number of nuclei in nuclear extracts (input) and flow-through supernatant after IP (unbound) were loaded for immunoblotting. The elution fraction for immunoblotting with anti-GFP or anti-HA antibody was ~20 times more concentrated than the nuclear extract, and that of anti-AtCDC5 antibody was ~40 times more concentrated. Protein from MOS4-HA (*A*) and PRL1-HA (*B*) transgenic plants were used as negative controls in anti-GFP IPs.

notypes (data not shown). Using an IP-Western procedure similar to what was done with MOS4, it was demonstrated that AtCDC5 coimmunoprecipitated with PRL1-GFP (Fig. 4B). Co-IP with HA column yielded similar results (data not shown).

A mutation in AtCDC5 *suppresses* snc1

To determine if AtCDC5 is also involved in *snc1*-dependent signaling, a T-DNA insertion line (SAIL_207_F03) in the 5′-untranslated region (UTR) of *AtCDC5* (At1g09770) was obtained from the *Arabidopsis* Biological Resource Center (ABRC). The morphological phenotypes of *Atcdc5-1* were very similar to *mos4-1*; like *mos4-1*, *Atcdc5-1* flowered late, but was slightly smaller than *mos4-1* and exhibited complete male sterility (Supplementary Fig. 2). All of these phenotypes were complemented by a genomic clone of wild-type *AtCDC5* (data not shown). The mutation in *AtCDC5* partially suppressed *snc1* morphology, as the *Atcdc5-1 snc1* double-mutant plants grew bigger and had leaves that were less curly than in *snc1* (Fig. 5A). *Atcdc5-1 snc1* plants also partially suppressed *PR* gene expression (Fig. 5B) and supported higher bacterial growth than *snc1* (Fig. 5C). Thus, the mutation in *AtCDC5* suppressed enhanced disease resistance responses in *snc1* similar to mutations in *MOS4*. Subsequently, a second T-DNA insertion line (GABI_278B09) in an exon of *AtCDC5* (*Atcdc5-2*) was obtained and determined to be allelic to *Atcdc5-1*. *Atcdc5-2* also suppressed *snc1* (data not shown).

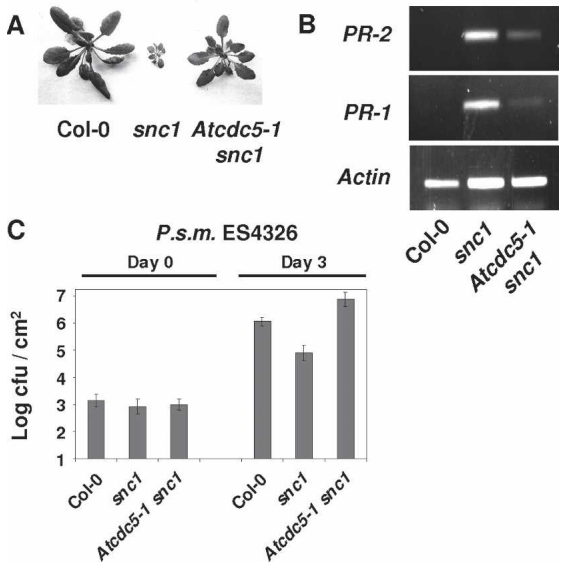

Figure 5. Suppression of *snc1* by *Atcdc5-1*. (*A*) Phenotypes of Col-0, *snc1*, and *Atcdc5-1 snc1* plants showing suppression of *snc1* morphology by *Atcdc5-1*. Image is of representative plants. (*B*) *PR-2* (*BGL2*) and *PR-1* gene expression in Col-0, *snc1*, and *Atcdc5-1 snc1*. RNA was extracted from 3-wk-old plants grown on MS medium and reverse-transcribed to obtain total cDNA. The cDNA samples were normalized by real-time PCR using an *Actin 1* probe. *PR-1*, *PR-2*, and *Actin 1* were amplified by 27 cycles of PCR using equal amounts of total cDNA. PCR products were analyzed by agarose gel electrophoresis and ethidium bromide staining. The results reported are representative of several experiments. (*C*) *Atcdc5-1* suppresses enhanced resistance of *snc1* to virulent *P.s.m.* ES4326, after infiltration at OD_{600} = 0.001. The experiment was repeated twice with similar results.

MOS4, AtCDC5, and PRL1 are all required for basal defense

Three T-DNA insertions in *PRL1* were obtained from the ABRC. The morphological phenotypes of all three *prl1* alleles were identical to each other and similar to those of *mos4-1* and *Atcdc5-1*, except with no flowering time defect (Supplementary Fig. 2). In addition, a genomic clone of wild-type *PRL1* was able to fully complement *prl1* phenotypes in 20 out of 22 transgenic T1 plants (data not shown). Because *PRL1* and *SNC1* are very closely linked on chromosome 4, we were unsuccessful in identifying the *prl1 snc1* double mutant (data not shown).

Since homologs of MOS4, AtCDC5, and PRL1 all belong to the NTC in fission yeast and human, an immediate question is whether or not these three genes affect innate immunity in a similar manner. We tested the *mos4*, *Atcdc5*, and *prl1* single mutants for EDS to a subclinical concentration of the virulent bacterial pathogen *P.s.m.* ES4326. At 3 DPI, disease symptoms were evident in infected leaves of all three mutants and absent in the wild-type Col-0 control (data not shown). Furthermore, *mos4* alleles and *Atcdc5-1* consistently supported ~25- to 50-fold more bacterial growth than wild-type plants, while *prl1* alleles supported an even higher titer of bacteria (at least 10-fold further) in the infected leaves, indicating that MOS4, AtCDC5, and PRL1 are all required for basal resistance to *P.s.m.* ES4326 (Fig. 6A). Similarly, *mos4*, *Atcdc5*, and *prl* mutants exhibited enhanced susceptibility to other virulent pathogens such as *H.p.* Noco2 (data not shown) and *Pseudomonas syringae* pv *tomato* (*P.s.t.*) DC3000 (Supplementary Fig. 9A).

Since alleles of *prl1* are consistently more susceptible than alleles of *mos4* or *Atcdc5* (Fig. 6A), we crossed *mos4-1* with *Atcdc5-1* or *prl1-1* to screen for respective double mutants to see if each putative complex member contributes quantitatively to plant immunity. We were unable to identify a *mos4-1 Atcdc5-1* or *mos4-1 prl1-1* double-mutant plant in the F_2 generation. After selecting several F_2 plants homozygous for one mutation and heterozygous for the other by PCR, for both mutant combinations, we were unable to find double-homozygous plants in several F_3 populations of >100 plants. This strongly suggests that *mos4-1 Atcdc5-1* and *mos4-1 prl1-1* double-homozygous plants are probably lethal.

To assay whether these mutants affected *PR* gene expression during pathogen infection, *mos4-1*, *Atcdc5-1*, *prl1-1* and *npr1-1* plants were inoculated with a clinical dose of virulent *P.s.m.* ES4326 and the RNA was extracted after 24 h. Real-time RT–PCR revealed that *PR-1* gene expression was absent in *npr1-1* and much lower in the other mutants tested, compared with wild type (Supplementary Fig. 9B).

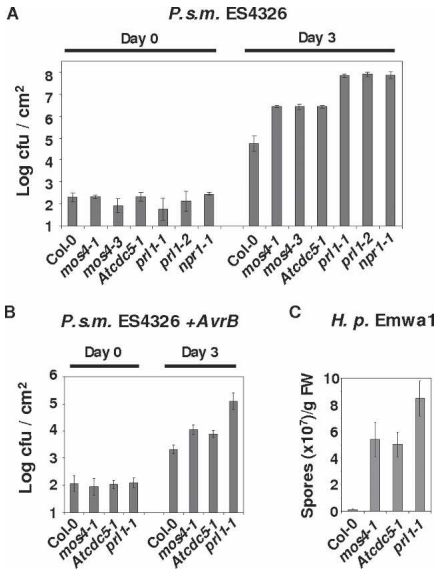

Figure 6. Characterization of *mos4*, *Atcdc5*, and *prl1* in innate immunity. (*A*) Growth of *P.s.m.* ES4326 in Col-0, *mos4-1*, *mos4-3*, *Atcdc5-1*, *prl1-1*, *prl1-2*, and *npr1-1* single mutants after infiltration at OD_{600} = 0.0001. (*B*) Growth of avirulent *P.s.m.* ES4326-expressing *AvrB*. The log-transformed values presented are averages of four replicates ± SD. (*C*) Growth of avirulent *H.p.* Emwa1 on Col-0, *mos4-1*, *Atcdc5-1*, and *prl1-1* plants. Results are averages of six replicates ± SD. The experiments were repeated three times with similar results.

Is enhanced susceptibility to *P.s.m* ES4326 in these mutants caused by a deficiency in SA accumulation? To answer this question, we inoculated quarter leaves of Col-0, *mos4-1*, *Atcdc5-1*, and *prl1-1* with a high dose of avirulent *P.s.m*. ES426 carrying *AvrB* and collected the remaining leaf tissue 24 h later, at which time macroscopic cell death was observed. Total SA was extracted and measured in pathogen- versus mock-inoculated leaves. As seen in Supplementary Figure 5, SA was able to accumulate in all three mutants as in wild type in response to an avirulent pathogen, indicating that MOS4 and its interacting partners do not contribute to SA accumulation after infection.

To test if defects in MOS4, AtCDC5, or PRL1 affected nonhost resistance, we inoculated plants with a high dose of *P.s.t.* DC3000 lacking *HrpA*, which encodes a pilus subunit required for the secretion of effectors into the host cytoplasm, and assayed for bacterial growth (Supplementary Fig. 10A). At 4 DPI, there was insignificant growth of *P.s.t.* DC3000 *hrpA*$^-$ in wild-type and all three mutant plants, suggesting that no defect in nonhost resistance exists as in mutants such as *nho1* (Lu et al. 2001). We also tested the response of these mutants to a model pathogen-associated molecular pattern (PAMP), flg22, the peptide epitope of the bacterial flagellin protein that is recognized by the receptor-like kinase FLS2 (Felix et al. 1999; Gomez-Gomez et al. 1999). The defense responses mediated by FLS2 are associated with a measurable inhibition of seedling growth upon prolonged treatment with flg22 (Gomez-Gomez et al. 1999). Ten days after application of 20 µM flg22, seedling growth inhibition was similar in wild-type Col-0 and in *mos4-1*, *Atcdc5-1*, and *prl1-1* seedlings compared with growth in media alone (Supplementary Fig. 10B,C). In addition, injection of flg22 peptide followed by inoculation with *P.s.t.* DC3000 24 h later resulted in enhanced resistance and consequently lower bacterial growth at 2 DPI versus mock treatment in wild type and in all three mutants (Supplementary Fig. 10D). The *Arabidopsis* ecotype Wassilewskija-0 (WS-0), which has a natural defect in *FLS2*, and the *fls2* mutant in Col-0 were not responsive to flg22 in either experiment, as expected. These data suggest that response to flg22 is not compromised in *mos4-1*, *Atcdc5-1*, or *prl1-1*, although we cannot exclude the possibility that these mutants are compromised in an FLS2-independent response to other PAMPs.

MOS4, AtCDC5, and PRL1 are all essential for R-protein-mediated resistance

To test if the *mos4*, *Atcdc5*, and *prl1* mutations affect resistance mediated by R proteins other than SNC1, we tested several *Avr-R* combinations that result in incompatible interactions in *Arabidopsis*. At least two distinct disease resistance pathways have been described for *Arabidopsis*, governed by R-protein structural type rather than pathogen class (Aarts et al. 1998). The *R* genes *RPM1* (CC-NB-LRR type) and *RPP4* (TIR-NB-LRR type) confer resistance to bacterial and oomycete pathogens expressing specific cognate avirulence gene products (Grant et al. 1995; van der Biezen et al. 2002). *mos4-1*, *Atcdc5-1*, and *prl1-1* were more susceptible than wild type to both avirulent *P.s.m*. ES4326 carrying *AvrB* (RPM1-dependent resistance) and avirulent *H.p.* Emwa1 (RPP4-dependent resistance), based on higher growth of pathogens in both mutants (Fig. 6B,C). Unlike EDS1 and NDR1, which are specifically required for signaling of TIR-NB-LRR and CC-NB-LRR-type R proteins, respectively, MOS4, AtCDC5, and PRL1 are required for resistance mediated by both types of R proteins. Other proteins required for both TIR and CC-NB-LRR R proteins include SGT1b, RAR1, and HSP90, which seem to function in stabilization of the R-protein complex rather than in downstream signaling (Austin et al. 2002; Azevedo et al. 2002; Hubert et al. 2003).

Because mutants in *MOS4*, *AtCDC5*, and *PRL1* exhibit very similar phenotypes and defects in basal and specific defense responses, MOS4 and PRL1 directly interact with AtCDC5, and homologs of all three proteins belong to the NTC in humans and yeast, we speculate that these three proteins must function together in a multiprotein complex orthologous to the human NTC to regulate innate immunity in *Arabidopsis*. We term this multiprotein assembly the MOS4-Associated Complex (MAC).

Discussion

The Arabidopsis *MAC and RNA splicing*

It is reasonable to propose that the *Arabidopsis* MAC is orthologous to the NTC, which has been indicated to be

essential for splicing (Ajuh et al. 2001), based on studies in human and yeast. Since the majority of plant genes are interrupted by noncoding introns, a defect in general splicing of mRNA would likely result in catastrophic phenotypes or death. For example, several general splicing mutants are embryo lethal, based on data in the SeedGenes database (Tzafrir et al. 2003). Since mutations in *MOS4*, *PRL1*, or *AtCDC5* alone only have minor effects on the plants' normal growth and development, it is unlikely that the protein products of these genes are essential components of splicing. However, since *mos4-1 Atcdc5-1* and *mos4-1 prl1-1* double-homozygous mutants seem to be lethal, MAC as a whole could be required for an essential process, such as spliceosome assembly, as suggested from studies in yeast and humans (Tarn et al. 1993; Grillari et al. 2005)

In some instances, the splicing machinery can process the same pre-mRNA differently by selectively joining different exons or retaining specific introns. This alternative splicing potentially leads to the generation of structurally and/or functionally distinct proteins. Several TIR-NB-LRR-class *R* genes, along with TLRs in animals, are alternatively spliced, although the functional relevance is unknown (Jordan et al. 2002). For example, the stoichiometry of two tobacco *N*-gene-derived splice variants undergoes a 50-fold change in their relative molar ratios upon pathogen challenge (Dinesh-Kumar and Baker 2000). The *Arabidopsis R* gene *RPS4* produces alternative transcripts with truncated ORFs. The dominant alternative *RPS4* transcripts are generated by retention of intron 3 or introns 2 and 3, which contain in-frame stop codons and lie downstream from the NB-encoding exon—the combined presence of regular and alternative *RPS4* transcripts is necessary for RPS4 function in resistance (Zhang and Gassmann 2003).

Although the functional relevance of alternative splicing in innate immunity is unknown, the association of the NTC with the spliceosome led us to hypothesize that MOS4, AtCDC5, and PRL1 may be involved in alternative splicing. We tested several alternatively spliced genes, including *RPS4*, *AtSRp30*, *AtSRp34*, *U1snRNP*, *ANP1*, and *POT1*, for relative levels of transcript variants of each in the *mos4-1*, *Atcdc5-1*, and *prl1-1* mutants by real-time RT–PCR. In all cases, there were no significant differences in transcript variant levels between wild type and the *mos4-1*, *Atcdc5*, and *prl1* mutants (Fig. 7). Our data suggest that MOS4, AtCDC5, and PRL1, while possibly associated with a spliceosome complex, are individually not involved in general or alternative RNA splicing. We cannot discount the possibility that the intact MAC complex is required for spliceosome assembly as the NTC is in yeast, or that the individual members regulate the splicing of an unknown RNA species.

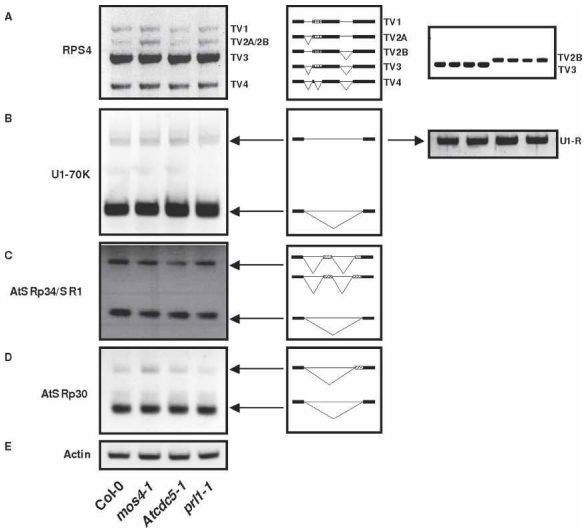

Figure 7. Levels of transcript variants in several alternatively spliced genes in Col-0, *mos4-1*, *Atcdc5-1*, and *prl1-1*. RNA was extracted from leaves and reverse-transcribed to obtain total cDNA. The cDNA samples were normalized by real-time PCR using an *Actin 1* probe. PCR products were analyzed by agarose gel electrophoresis and ethidium bromide staining. Schematic diagrams of alternative splicing are shown at *right*. (*A*) Alternative splicing of *RPS4* results in five transcript variants (TV) of different sizes, as determined by PCR using nested primers as previously described (Zhang and Gassmann 2003). In this case, TV1 retained both introns in the schematic diagram, whereas TV2 was composed of two transcript species of similar size lacking either the first intron (TV2A) or second intron (TV2B). TV3 corresponds to the regular transcript with both introns removed. An additional cryptic intron was spliced out in TV4. Primers were designed across intron/exon boundaries to specifically amplify TV3 and TV2B from diluted PCR products of the distal nested primers. As determined by real-time PCR and shown in the far *right* panel, there was no significant difference in relative abundance of either alternatively spliced *RPS4* transcripts between Col-0, *mos4-1*, *Atcdc5-1*, and *prl1-1*. (*B*) Alternative splicing of *U1-70K* featuring intron retention (Savaldi-Goldstein et al. 2003). Primers were designed across intron/exon boundaries to specifically amplify the retained intron transcript of *U1-70K*, and relative abundance in Col-0 and MAC mutants was assayed by real-time PCR; as determined by real-time PCR and shown in the far *right* panel, there was no significant difference in relative abundance of retained intron *U1-70K* transcript (U1-R). (*C*) *AtSRp34/SR1* showing 5′ and 3′ alternative splicing (Savaldi-Goldstein et al. 2003). (*D*) *AtSRp30* showing 5′ and 3′ alternative splicing (Savaldi-Goldstein et al. 2003). (*E*) *Actin* control, 30 cycles. Alternative splicing of ANP1 and POT1 (Tani and Murata 2005) in the MAC mutants resulted in similar patterns (data not shown).

What is the function of the MAC and NTC in plants and animals?

In human cells, in addition to being associated physically with the spliceosome, the MOS4 ortholog BCAS2 and hCDC5L have been reported to have roles in cancer malignancy, apoptosis, estrogen receptor-mediated transcription, and DNA repair (Lee et al. 2002; Maass et al. 2002; Mahajan and Mitchell 2003; Qi et al. 2005; N. Zhang et al. 2005). The detailed biochemical and biological function of BCAS2, hCDC5L, and other members of the human NTC remains elusive in part because of the challenge of genetic analysis at a whole-organism level. Our analysis of the *mos4*, *Atcdc5*, and *prl1* mutants highlights the advantage of using *Arabidopsis* genetics

to dissect biological processes unambiguously and provides an exceptional opportunity to define the function of the NTC. The NTC is an evolutionarily conserved protein complex that interacts in an as-yet-undetermined way with the spliceosome. Future yeast two-hybrid or proteomic efforts using MOS4 epitope-tagged lines will enable us to identify more *Arabidopsis* components of the complex and analyze their functions using reverse genetic approaches. It is important to note that, although we show that MOS4, AtCDC5, and PRL1 are required for innate immunity, there is as of yet no direct evidence that their interactions are required for innate immunity.

Defects of *MOS4*, *PRL1*, and *AtCDC5* are confined to innate immunity, flowering time, and fertility, indicating that targets of MAC/AtNTC regulation are limited. Members of the human NTC include PSO4, an E3 ubiquitin ligase, hCDC5L, a transcription factor, and several proteins with protein interaction domains (e.g., BCAS2 and PLRG1), hinting at a complex regulatory module (Loscher et al. 2005; N. Zhang et al. 2005). Since AtCDC5 has sequence-specific DNA-binding capability (Hirayama and Shinozaki 1996), the simplest and most likely explanation is that the MAC fulfills its role through regulating transcription of downstream target genes. The spliceosome may assist the NTC in fulfilling this regulatory function. Alternatively, the NTC may merely colocalize with the spliceosome based on its association with the nuclear structure. A third possibility could be that the MAC contributes to regulatory processes mediated by microRNA (miRNA) and small interfering RNA (siRNA), both of which have been shown recently to be involved in the regulation of development and innate immunity (Katiyar-Agarwal et al. 2006; Navarro et al. 2006). It is interesting that endogenous small RNAs are also involved in the regulation of developmental processes, such as flowering time via FLC (Swiezewski et al. 2007). Future detailed genetic and biochemical analysis using *Arabidopsis mac* mutants will help reveal these underlying mechanisms. It will also be very interesting to test whether the NTC functions in the regulation of animal innate immunity.

Materials and methods

Mutant screen and characterization of mos4

All plants were grown under a 16 h light/8 h dark regime. The *mos* suppressor screen and GUS staining protocol have been described previously (Zhang and Li 2005).

RNA used for gene expression analysis was extracted from 3-wk-old seedlings grown on Murashige and Skoog (MS) medium using the Totally RNA kit (Ambion). RT was carried out using the RT-for-PCR kit (Clontech). Real-time PCR was performed using the QuantiTect SYBR Green PCR kit (Qiagen). The primers used for amplification of *Actin1*, *PR-1*, and *PR-2* were described previously (Zhang et al. 2003).

SA was extracted and measured from 4-wk-old soil-grown leaves using a previously described procedure (Li et al. 1999), proportionally scaled down using ~100 mg of tissue per sample. Four-week-old soil-grown *Arabidopsis* plants were infected with *P.s.m.* ES4326. Leaf discs of 0.32 cm^2 were taken with a standard paper hole-punch. Infection of plants with *H.p.* Noco2 and *H.p.* Emwa1 was performed on 2-wk-old seedlings. These procedures were as described (Li et al. 1999).

Subcellular localization of MOS4

The Gateway LR Clonase enzyme (Invitrogen) was used to catalyze the recombination between *pENTR-gMOS4* (see the Supplemental Material) and a destination vector that is a modified binary vector with TOPO destination recombination sites with GFP fused in-frame to the C terminus. The *MOS4-GFP* expression clone was sequenced to confirm in-frame fusion and a lack of PCR errors. Transgenic plants were selected on MS medium containing 75 µg/mL kanamycin. Guard cells of transgenic seedlings were examined for GFP fluorescence as described previously (Y. Zhang et al. 2005).

Yeast two-hybrid assay

We used the GAL4-based Y2H system described previously (Kohalmi et al. 1998). Briefly, a GAL4(DB)-cMOS4 construct (see the Supplemental Material) was introduced into Y2H strain YPB2 and used to screen an *Arabidopsis* cDNA expression library (kindly provided by William Crosby) encoding *Arabidopsis* proteins as C-terminal fusions to the GAL4 transcription activation domain (TA). Putative MOS4-interacting proteins were selected on the basis of histidine prototrophy and further screened on the basis of *lacZ* expression.

In planta protein interaction assay

Nuclear extracts (see the Supplemental Material) of transgenic *mos4-1* complemented with *gMOS4-GFP* or *gMOS4-HA* and *prl1-1* complemented with *gPRL1-GFP* or *gPRL1-HA* were mixed with 50 µL of Anti-GFP or Anti-HA MicroBeads (Miltenyi Biotec) to magnetically absorb the epitope-tagged target protein. After an overnight incubation on ice, the MicroBead-bound target protein was magnetically precipitated on columns according to the manufacturer's instructions (µMACs; Miltenyi Biotec) for subsequent immunoblotting analysis. Immunoprecipitated proteins eluted from the column were detected with anti-GFP or anti-HA antibody, respectively, and the coimmunoprecipitated endogenous AtCDC5 protein was detected by a polyclonal antibody raised against the N-terminal 144-amino-acid fragment of AtCDC5.

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References

Aarts, N., Metz, M., Holub, E., Staskawicz, B.J., Daniels, M.J., and Parker, J.E. 1998. Different requirements for EDS1 and

NDR1 by disease resistance genes define at least two R gene-mediated signaling pathways in *Arabidopsis*. *Proc. Natl. Acad. Sci.* **95:** 10306–10311.

Ajuh, P., Kuster, B., Panov, K., Zomerdijk, J.C., Mann, M., and Lamond, A.I. 2000. Functional analysis of the human CDC5L complex and identification of its components by mass spectrometry. *EMBO J.* **19:** 6569–6581.

Ajuh, P., Sleeman, J., Chusainow, J., and Lamond, A.I. 2001. A direct interaction between the carboxyl-terminal region of CDC5L and the WD40 domain of PLRG1 is essential for pre-mRNA splicing. *J. Biol. Chem.* **276:** 42370–42381.

Akira, S., Uematsu, S., and Takeuchi, O. 2006. Pathogen recognition and innate immunity. *Cell* **124:** 783–801.

Austin, M.J., Muskett, P., Kahn, K., Feys, B.J., Jones, J.D., and Parker, J.E. 2002. Regulatory role of SGT1 in early R gene-mediated plant defenses. *Science* **295:** 2077–2080.

Ausubel, F.M. 2005. Are innate immune signaling pathways in plants and animals conserved? *Nat. Immunol.* **6:** 973–979.

Azevedo, C., Sadanandom, A., Kitagawa, K., Freialdenhoven, A., Shirasu, K., and Schulze-Lefert, P. 2002. The RAR1 interactor SGT1, an essential component of R gene-triggered disease resistance. *Science* **295:** 2073–2076.

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.

Belkhadir, Y., Subramaniam, R., and Dangl, J.L. 2004. Plant disease resistance protein signaling: NBS-LRR proteins and their partners. *Curr. Opin. Plant Biol.* **7:** 391–399.

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.

Dinesh-Kumar, S.P. and Baker, B.J. 2000. Alternatively spliced N resistance gene transcripts: Their possible role in tobacco mosaic virus resistance. *Proc. Natl. Acad. Sci.* **97:** 1908–1913.

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

Eckmann, L. and Karin, M. 2005. NOD2 and Crohn's disease: Loss or gain of function? *Immunity* **22:** 661–667.

Felix, G., Duran, J.D., Volko, S., and Boller, T. 1999. Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *Plant J.* **18:** 265–276.

Gomez-Gomez, L., Felix, G., and Boller, T. 1999. A single locus determines sensitivity to bacterial flagellin in *Arabidopsis thaliana*. *Plant J.* **18:** 277–284.

Grant, M.R., Godiard, L., Straube, E., Ashfield, T., Lewald, J., Sattler, A., Innes, R.W., and Dangl, J.L. 1995. Structure of the *Arabidopsis* RPM1 gene enabling dual specificity disease resistance. *Science* **269:** 843–846.

Grillari, J., Ajuh, P., Stadler, G., Loscher, M., Voglauer, R., Ernst, W., Chusainow, J., Eisenhaber, F., Pokar, M., Fortschegger, K., et al. 2005. SNEV is an evolutionarily conserved splicing factor whose oligomerization is necessary for spliceosome assembly. *Nucleic Acids Res.* **33:** 6868–6883.

Hirayama, T. and Shinozaki, K. 1996. A cdc5$^+$ homolog of a higher plant, *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci.* **93:** 13371–13376.

Hubert, D.A., Tornero, P., Belkhadir, Y., Krishna, P., Takahashi, A., Shirasu, K., and Dangl, J.L. 2003. Cytosolic HSP90 associates with and modulates the *Arabidopsis* RPM1 disease resistance protein. *EMBO J.* **22:** 5679–5689.

Jordan, T., Schornack, S., and Lahaye, T. 2002. Alternative splicing of transcripts encoding Toll-like plant resistance proteins—What's the functional relevance to innate immunity? *Trends Plant Sci.* **7:** 392–398.

Katiyar-Agarwal, S., Morgan, R., Dahlbeck, D., Borsani, O., Villegas Jr., A., Zhu, J.K., Staskawicz, B.J., and Jin, H. 2006. A pathogen-inducible endogenous siRNA in plant immunity. *Proc. Natl. Acad. Sci.* **103:** 18002–18007.

Kohalmi, S.E., Reader, L.J.W., Samach, A., Nowak, J., Haughn, G.W., and Crosby, W.L. 1998. Identification and characterization of protein interactions using the yeast 2-hybrid system. In *Plant molecular biology manual M1* (eds. S.B. Gelvin and R.A. Schilperoort), pp. 1–30. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Lee, S., Ha, S., Chung, M., Kim, Y., and Choi, Y. 2002. Mouse DAM1 regulates pro-apoptotic activity of BLK in mammary epithelial cells. *Cancer Lett.* **188:** 121–126.

Li, X., Zhang, Y., Clarke, J.D., Li, Y., and Dong, X. 1999. Identification and cloning of a negative regulator of systemic acquired resistance, SNI1, through a screen for suppressors of npr1-1. *Cell* **98:** 329–339.

Loscher, M., Fortschegger, K., Ritter, G., Wostry, M., Voglauer, R., Schmid, J.A., Watters, S., Rivett, A.J., Ajuh, P., Lamond, A.I., et al. 2005. Interaction of U-box E3 ligase SNEV with PSMB4, the β7 subunit of the 20 S proteasome. *Biochem. J.* **388:** 593–603.

Lu, M., Tang, X.Y., and Zhou, J.M. 2001. Arabidopsis NHO1 is required for general resistance against *Pseudomonas* bacteria. *Plant Cell* **13:** 437–447.

Maass, N., Rosel, F., Schem, C., Hitomi, J., Jonat, W., and Nagasaki, K. 2002. Amplification of the BCAS2 gene at chromosome 1p13.3-21 in human primary breast cancer. *Cancer Lett.* **185:** 219–223.

Mahajan, K.N. and Mitchell, B.S. 2003. Role of human Pso4 in mammalian DNA repair and association with terminal deoxynucleotidyl transferase. *Proc. Natl. Acad. Sci.* **100:** 10746–10751.

Navarro, L., Dunoyer, P., Jay, F., Arnold, B., Dharmasiri, N., Estelle, M., Voinnet, O., and Jones, J.D. 2006. A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. *Science* **312:** 436–439.

Nemeth, K., Salchert, K., Putnoky, P., Bhalerao, R., Koncz-Kalman, Z., Stankovic-Stangeland, B., Bako, L., Mathur, J., Okresz, L., Stabel, S., et al. 1998. Pleiotropic control of glucose and hormone responses by PRL1, a nuclear WD protein, in *Arabidopsis*. *Genes & Dev.* **12:** 3059–3073.

Neubauer, G., King, A., Rappsilber, J., Calvio, C., Watson, M., Ajuh, P., Sleeman, J., Lamond, A., and Mann, M. 1998. Mass spectrometry and EST-database searching allows characterization of the multi-protein spliceosome complex. *Nat. Genet.* **20:** 46–50.

Ohi, M.D. and Gould, K.L. 2002. Characterization of interactions among the Cef1p–Prp19p-associated splicing complex. *RNA* **8:** 798–815.

Philpott, D.J. and Girardin, S.E. 2004. The role of Toll-like receptors and Nod proteins in bacterial infection. *Mol. Immunol.* **41:** 1099–1108.

Qi, C., Zhu, Y.T., Chang, J., Yeldandi, A.V., Rao, M.S., and Zhu, Y.J. 2005. Potentiation of estrogen receptor transcriptional activity by breast cancer amplified sequence 2. *Biochem. Biophys. Res. Commun.* **328:** 393–398.

Savaldi-Goldstein, S., Aviv, D., Davydov, O., and Fluhr, R. 2003. Alternative splicing modulation by a LAMMER kinase impinges on developmental and transcriptome expression. *Plant Cell* **15:** 926–938.

Swiezewski, S., Crevillen, P., Liu, F., Ecker, J.R., Jerzmanowski, A., and Dean, C. 2007. Small RNA-mediated chromatin silencing directed to the 3′ region of the *Arabidopsis* gene

encoding the developmental regulator, FLC. *Proc. Natl. Acad. Sci.* **104:** 3633–3638.

Tani, A. and Murata, M. 2005. Alternative splicing of Pot1 (Protection of telomere)-like genes in *Arabidopsis thaliana*. *Genes Genet. Syst.* **80:** 41–48.

Tarn, W.Y., Lee, K.R., and Cheng, S.C. 1993. The yeast PRP19 protein is not tightly associated with small nuclear RNAs, but appears to associate with the spliceosome after binding of U2 to the pre-mRNA and prior to formation of the functional spliceosome. *Mol. Cell. Biol.* **13:** 1883–1891.

Tsai, W.Y., Chow, Y.T., Chen, H.R., Huang, K.T., Hong, R.I., Jan, S.P., Kuo, N.Y., Tsao, T.Y., Chen, C.H., and Cheng, S.C. 1999. Cef1p is a component of the Prp19p-associated complex and essential for pre-mRNA splicing. *J. Biol. Chem.* **274:** 9455–9462.

Tzafrir, I., Dickerman, A., Brazhnik, O., Nguyen, Q., McElver, J., Frye, C., Patton, D., and Meinke, D. 2003. The *Arabidopsis* SeedGenes Project. *Nucleic Acids Res.* **31:** 90–93.

van der Biezen, E.A., Freddie, C.T., Kahn, K., Parker, J.E., and Jones, J.D. 2002. *Arabidopsis* RPP4 is a member of the RPP5 multigene family of TIR-NB-LRR genes and confers downy mildew resistance through multiple signalling components. *Plant J.* **29:** 439–451.

Zhang, X.C. and Gassmann, W. 2003. RPS4-mediated disease resistance requires the combined presence of RPS4 transcripts with full-length and truncated open reading frames. *Plant Cell* **15:** 2333–2342.

Zhang, Y. and Li, X. 2005. *MOS3*, encoding a putative Nucleoporin 96, is required for both basal defense and constitutive resistance responses mediated by *snc1*. *Plant Cell* **17:** 1306–1316.

Zhang, Y., Goritschnig, S., Dong, X., and Li, X. 2003. A gain-of-function mutation in a plant disease resistance gene leads to constitutive activation of downstream signal transduction pathways in suppressor of npr1-1, constitutive 1. *Plant Cell* **15:** 2636–2646.

Zhang, N., Kaur, R., Lu, X., Shen, X., Li, L., and Legerski, R.J. 2005. The Pso4 mRNA splicing and DNA repair complex interacts with WRN for processing of DNA interstrand cross-links. *J. Biol. Chem.* **280:** 40559–40567.

Zhang, Y., Cheng, Y.T., Bi, D., Palma, K., and Li, X. 2005. MOS2, a protein containing G-patch and KOW motifs, is essential for innate immunity in *Arabidopsis thaliana*. *Curr. Biol.* **15:** 1936–1942.

Zhou, Z., Licklider, L.J., Gygi, S.P., and Reed, R. 2002. Comprehensive proteomic analysis of the human spliceosome. *Nature* **419:** 182–185.