Differential Roles of Two Homologous Cyclin-Dependent Kinase Inhibitor Genes in Regulating Cell Cycle and Innate Immunity in Arabidopsis[1][OPEN]

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Precise cell-cycle control is critical for plant development and responses to pathogen invasion. Two homologous cyclin-dependent kinase inhibitor genes, *SIAMESE* (*SIM*) and *SIM-RELATED 1* (*SMR1*), were recently shown to regulate Arabidopsis (*Arabidopsis thaliana*) defense based on phenotypes conferred by a *sim smr1* double mutant. However, whether these two genes play differential roles in cell-cycle and defense control is unknown. In this report, we show that while acting synergistically to promote endoreplication, *SIM* and *SMR1* play different roles in affecting the ploidy of trichome and leaf cells, respectively. In addition, we found that the *smr1-1* mutant, but not *sim-1*, was more susceptible to a virulent *Pseudomonas syringae* strain, and this susceptibility could be rescued by activating salicylic acid (SA)-mediated defense. Consistent with these results, *smr1-1* partially suppressed the dwarfism, high SA levels, and cell death phenotypes in *acd6-1*, a mutant used to gauge the change of defense levels. Thus, *SMR1* functions partly through SA in defense control. The differential roles of *SIM* and *SMR1* are due to differences in temporal and spatial expression of these two genes in Arabidopsis tissues and in response to *P. syringae* infection. In addition, flow-cytometry analysis of plants with altered SA signaling revealed that SA is necessary, but not sufficient, to change cell-cycle progression. We further found that a mutant with three *CYCD3* genes disrupted also compromised disease resistance to *P. syringae*. Together, this study reveals differential roles of two homologous cyclin-dependent kinase inhibitors in regulating cell-cycle progression and innate immunity in Arabidopsis and provides insights into the importance of cell-cycle control during host-pathogen interactions.

Properly controlled cell-cycle progression is critical for plant growth and development (Inzé and De Veylder, 2006; De Veylder et al., 2011; Polyn et al., 2015). The cell-cycle machinery has recently been shown to be important for plant defense (Bao et al., 2013; Chandran et al., 2014; Wang et al., 2014b; Bao and Hua, 2015). However, how the host cell-cycle machinery is modulated during host-pathogen interactions has not been completely understood.

Mitotic cell cycle is typically divided into five major phases: quiescent phase (G0), postmitotic interphase (G1), DNA synthesis phase (S), postsynthetic interphase (G2), and mitosis (M). Transitions between phases in the cell cycle are tightly regulated at checkpoints in plants, animals, and yeast (Harashima et al., 2013). Heterodimeric protein kinase complexes, which consist of catalytic subunits (cyclin-dependent kinases, or CDKs) and regulatory subunits (cyclins, or CYCs), are the main gatekeepers of the checkpoints. Activities of CYC/CDK complexes can be regulated at multiple levels (Potuschak and Doerner, 2001; Berckmans and De Veylder, 2009; Harashima et al., 2013). For instance, CYCs are prone to degradation via ubiquitin-mediated pathways involving at least two types of ubiquitin E3 ligases, the anaphase-promoting complex and the Skp1/Cullin/F-box related complex (Vodermaier, 2004; Heyman and De Veylder, 2012). Activities of CYC/CDK complexes can also be regulated by CDK-activating kinases and CDK inhibitors (CKIs; Sherr and Roberts, 1999; Umeda et al., 2005; De Clercq and Inzé, 2006). Arabidopsis (*Arabidopsis thaliana*) has two classes of CKIs, the Kip-related proteins (KRPs), which are similar to the mammalian Kip/Cip proteins (De

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H.L. conceived the research plans and wrote the article; S.H. performed most of the experiments; C.Z. and H.L. provided expression data with defense condition; M.G. and A.K. provided technical assistance to S.H.; and N.K., M.C., and J.C.L. provided scanning EM images and the trichome initiation data.

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Veylder et al., 2001), and SIAMESE/SIAMESE-RELATED (SIM/SMR) proteins, which are more distantly related to KRPs and are plant specific (Churchman et al., 2006; Peres et al., 2007).

Active CYC/CDK complexes can target downstream cell-cycle core components to trigger specific cell-cycle events. For instance, a CDKA/CYCD complex can phosphorylate the retinoblastoma-related protein (RBR), and such a modification releases the binding of RBR to the transcription factors E2F, subsequently activating E2F and promoting expression of genes necessary for the transition from G1 to S phase, while CYCA- and CYCB-containing CDK complexes trigger entry into mitosis. However, when activities of mitotic CYC/CDK complexes are suppressed, the cell cycle is reprogrammed to enter endoreplication in which multiple rounds of DNA replication occur without subsequent mitosis and cytokinesis (De Veylder et al., 2011). Endoreplication, also called endoreduplication, endocycling, or endoploidization, is a common variant of the cell cycle of many cell types during plant development (Bramsiepe et al., 2010; De Veylder et al., 2011).

Many studies have also linked cell-cycle control with plant responses to pathogen invasions. Pathogens, such as the bacteria *Pseudomonas syringae* and *Xanthomonas citri*, the actinomycete *Rhodococcus fascians*, the fungal pathogen powdery mildew, and nematodes, can induce endoreplication in some cells at or near the infected loci of host tissue (Swarup et al., 1991; Chandran et al., 2010; Wildermuth, 2010; de Almeida Engler and Gheysen, 2013; Hamdoun et al., 2013). Consistent with pathogen-induced endoreplication in the host, some pathogen effectors are known to interact with host cell-cycle components, either through direct physical protein-protein interaction and/or through modulating expression of genes critical for cell-cycle progression (Kay et al., 2007; Mukhtar et al., 2011). Expression of several core cell-cycle genes of plants was also known to be induced or suppressed during infections (Niebel et al., 1996; de Almeida Engler et al., 1999; Favery et al., 2002; Ascencio-Ibáñez et al., 2008; Chandran et al., 2010). In addition, several core cell-cycle genes were shown to be important for plant defense against pathogens (Favery et al., 2002; Depuydt et al., 2009a, 2009b; Bao et al., 2013; Chandran et al., 2014; Wang et al., 2014b). Thus, cell-cycle control is intimately interconnected with plant defense responses.

Among the defense-related core cell-cycle genes, *SIM* and *SMR1* belong to a plant-specific CKI family with 17 members, functions of which have not been well understood (Yi et al., 2014). *SIM* is the founding member of the family and was previously studied for its role in trichome development and endoreplication in trichome cells (Walker et al., 2000; Churchman et al., 2006). Leaves of wild-type plants have unicellular trichomes, and each has three to four branches and a single nucleus containing 16 to 32 C-value of DNA. In contrast, most trichomes of *sim* loss-of-function mutants are multicellular and consist of up to 15 cells with significantly reduced nuclear DNA content (Walker et al., 2000; Churchman et al., 2006). Thus, *SIM* was proposed to be a positive regulator of endoreplication in trichomes. *SMR1*, also called *LOSS OF GIANT CELLS FROM ORGANS*, is the closest homolog of *SIM* in the *SMR* family (Roeder et al., 2010). *SMR1* affects the formation of giant endoreplicated pavement cells on Arabidopsis sepals (Roeder et al., 2010). However, the role of *SMR1* in trichome development has not been well studied. Recently, a *sim smr1* double mutant was shown to have compromised responses to *P. syringae* and the oomycete pathogen *Hyaloperonospera arabidopsidis* (Wang et al., 2014b). However, the individual single mutants were not analyzed in this study. It is likely that the observed defense phenotypes in the *sim smr1* double mutant are due to the synergistic effect of the two genes. It is also possible that these two genes could play differential roles in affecting Arabidopsis defense. Therefore, it is important to further elucidate the roles of *SIM* and *SMR1* in regulating defense and cell-cycle progression in order to gain better understanding of functions of these two genes.

In this report, we analyzed mutants impaired in *SIM*, *SMR1*, or both genes for cell-cycle and defense phenotypes. We found that *SIM* promotes endoreplication predominately in the trichome while *SMR1* in non-trichome leaf cells. The two genes also act synergistically to affect endoreplication in leaf cells. In addition, we found that that *SMR1* plays a greater role than *SIM* in regulating resistance to *P. syringae*. The defense function of *SMR1* is at least partly through signaling mediated by salicylic acid (SA). Such differential roles of *SIM* and *SMR1* are most likely due to differential expression of these genes in Arabidopsis tissues and in response to pathogen infection. On the other hand, cell ploidy analyses of SA mutants and plants treated with an SA analog indicate that SA signaling is necessary, but not sufficient, to disrupt cell-cycle progression. Interestingly, similar to *sim smr1* that has reduced endoreplication, a mutant with three *CYCD3* genes disrupted that has increased endoreplication also showed compromised disease resistance to *P. syringae*. In addition, we found that expression of *SMR1* and several cell-cycle genes were suppressed under defense conditions, suggesting a negative feedback regulation between cell-cycle progression and defense activation. Together, our study reveals differential roles of two homologous *CKI*s in regulating cell-cycle progression and innate immunity in Arabidopsis and provides important insight into the role of cell cycle in affecting host-pathogen interactions.

RESULTS

The *SIM* and *SMR1* Genes Act Synergistically to Affect Trichome Development

Wild-type Columbia-0 (Col-0) plants form single trichomes, each consisting of a single cell with multiple branches. In contrast, most trichomes of the *sim-1* mutant are multicellular, with about 2.5 cells per trichome

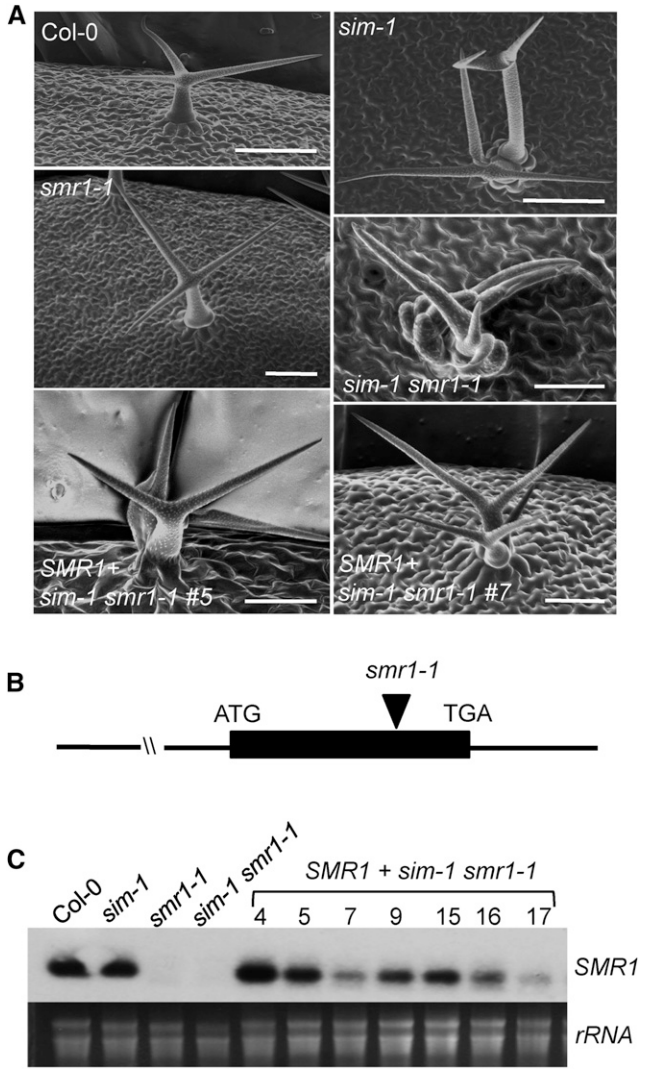

Figure 1. Rescue *sim-1 smr1-1*-conferred phenotypes by a *SMR1* genomic fragment. A, Scanning electron micrographs. Two-week-old plants were observed with a scanning electron microscope. Bars in Col-0, *sim-1*, and *smr1-1* are 100 μm. Bars in *sim-1 smr1-1* and the lines 5 and 7 are 50 μm. B, The *SMR1* construct used for the rescue experiment. The position of the *smr1-1* mutation was indicated. C, Expression of *SMR1* in transgenic *sim-1 smr1-1* plants. A construct containing a 2367 bp *SMR1* genomic fragment was used to transform the *sim-1 smr1-1* mutant, and independently transformed homozygous lines (4, 5, 7, 9, 15, 16, and 17) were obtained. Total RNA was extracted and analyzed by northern blotting. These experiments were repeated twice with similar results.

(Fig. 1A; Supplemental Figs. S1 and S2; Table I; Walker et al., 2000; Churchman et al., 2006). Trichomes of *sim-1* have reduced DNA content than those of Col-0, leading to the conclusion that SIM acts as a positive regulator of endoreplication in trichomes (Walker et al., 2000; Churchman et al., 2006). Among the SMR family members, SMR1 exhibits the highest homology to SIM with 62% identity at the amino acid level. However, a null mutation caused by a T-DNA insertion in the *SMR1* gene, *smr1-1*, did not affect trichome morphology. Like Col-0, the trichomes of *smr1-1* were unicellular (Fig. 1; Supplemental Figs. S1 and S2; Table I). On the other hand, the double mutant *sim-1 smr1-1* produced much smaller and deformed trichomes than *sim-1* along. A closer inspection revealed that trichomes of *sim-1 smr1-1* branched from the bases of the trichome initiation sites and contained more than twice the number of cells per initiation site as in *sim-1*. On the other hand, *smr1-1* and Col-0 had a similar number of cells per trichome initiation site (Fig. 1A; Table I).

We were able to rescue the *sim-1 smr1-1* mutant with a *SMR1* genomic fragment, including sequences of 1424 bp 5′ end upstream and 637 bp 3′ end downstream of the *SMR1* coding region (Fig. 1B). The transgenic *sim-1 smr1-1* plants expressed variable levels of the *SMR1* transgene and were rescued for the severe trichome phenotype, as shown in the two representative lines 5 and 7 (Fig. 1, A and C; Supplemental Fig. S2; Table I). These transgenic lines still showed the weaker multicellular trichome phenotype of the *sim-1* mutant, suggesting that increased *SMR1* expression did not affect the *sim-1* phenotype. Thus, *SIM* and *SMR1* may function differently in regulating trichome development. Together these data suggest that *SIM* plays a major role in regulating endoreplication of trichome cells and trichome development. While *SMR1* by itself may play a lesser role in regulating these processes, it could contribute synergistically to such function of *SIM*.

SIM and *SMR1* Act Synergistically to Affect Cell Ploidy in the Leaf

To further determine if *SIM* and *SMR1* affect cell-cycle progression in other cell types besides the trichome, we measured the ploidy of leaf cells using flow cytometry. We found that although showing strong trichome defects, the *sim-1* mutant did not affect overall cell ploidy pattern in the leaf, compared with Col-0 (Fig. 2A). The *smr1-1* mutant repeatedly showed lower cell ploidy, having increased 4C but decreased 16C cells. However, the difference between Col-0 and *smr1-1* was not statistically significant in our analysis, although it was reported so in a previous study (Roeder et al.,

Table I. *SMR1 rescued the multicellular phenotype of the sim-1 smr1-1 double mutant*

Nuclei/TIS, Nuclei per trichome initiation site, a measure of the number of cells per trichome initiation site. Statistical analysis was performed with one-way ANOVA. Letters indicate significant difference among the samples ($P < 0.05$).

Genotype	Nuclei/TIS	No. of TIS
Col-0	1.0 ± 0 [a]	50
sim-1	2.5 ± 1.1 [b]	50
smr1-1	1.0 ± 0 [a]	50
sim-1 smr1-1	5.9 ± 2.1 [c]	50
SMR1+sim-1 smr1-1 #5	2.2 ± 1.1 [b]	50
SMR1+sim-1 smr1-1 #7	2.8 ± 1.3 [b]	50

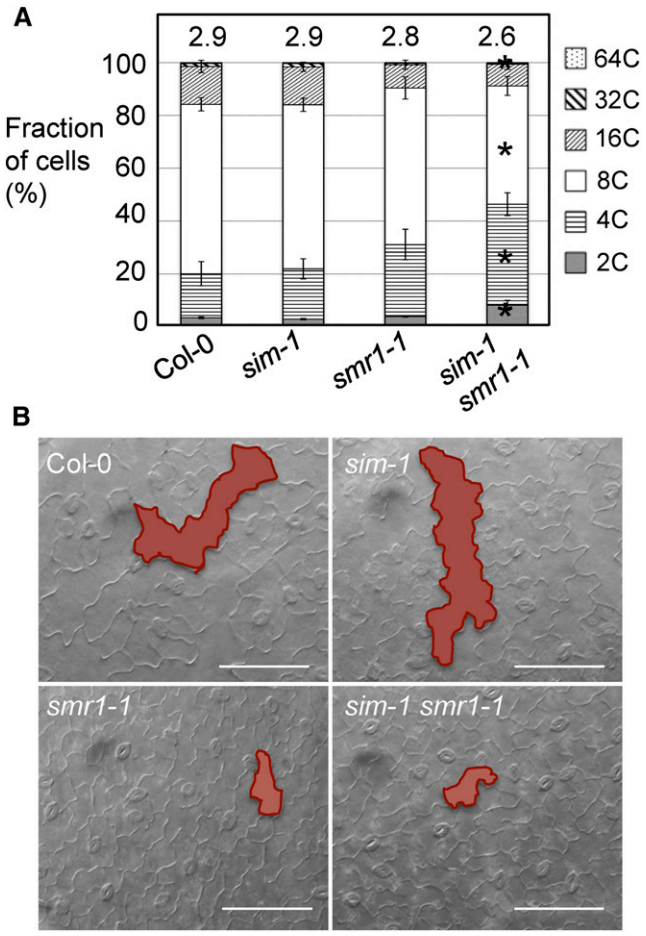

Figure 2. *SMR1* plays a stronger role than *SIM* in regulating ploidy of leaf cells. A, Analysis of leaf cell ploidy by flow cytometry. The fourth to sixth leaves of 25-d-old plants were collected for nuclei isolation, followed by flow cytometry analysis. Data represent the average of five experiments ± SE of mean. Statistical analysis was performed with Mann-Whitney test (http://vassarstats.net/). Asterisks indicate significant difference between samples of different genotypes in the same ploidy group ($P < 0.05$). Ploidy indices are shown above the bars. B, Epidermal cell morphology. The fourth to sixth leaves of 25-d-old plants were cleared overnight with ethanol and photographed using a dissecting microscope connected to a CCD camera. Highlighted shapes indicate typical pavement cells in each genotype. Note giant pavement cells in Col-0 and *sim-1*, but not in *smr1-1* and *sim-1 smr1-1*. Bars, 100 μm.

2010). The *sim-1 smr1-1* mutant, on the other hand, showed a significant increase in 2C and 4C cells but a decrease in 8C and 64C cells. *sim-1 smr1-1* also had significantly reduced ploidy index (2.6 ± 0.2), compared with those of *sim-1* (2.9 ± 0.1), *smr1-1* (2.8 ± 0.2), and Col-0 (2.9 ± 0.2).

Consistent with the change of cell ploidy detected by flow cytometry, we found that while both *sim-1* and Col-0 showed large pavement cells on the leaf surface, *smr1-1* and *sim-1 smr1-1* mutants lacked these giant cells (Fig. 2B). Together, these results suggest that *SMR1* plays a stronger role than *SIM* in regulating endoreplication of nontrichome leaf cells in Arabidopsis.

SMR1 Plays a Greater Role in Disease Resistance to *P. syringae*

Recently *SIM* and *SMR1* were shown to affect effector triggered immunity in response to *P. syringae* and *H. arabidopsidis* (Wang et al., 2014b). However, in this study only a *sim smr1* double mutant, but not the individual single mutants, were tested. Our data showing differential roles of *SIM* and *SMR1* in cell-cycle control prompted us to ask whether these two genes also play differential roles in defense control. To address this question, we infected *sim-1* and *smr1-1* mutants with the virulent strain *P. syringae* pv *maculicola* ES4326 DG3 (PmaDG3). We found that while *sim-1* had a similar level of bacterial growth as Col-0, *smr1-1* was more susceptible to *P. syringae* than Col-0 (Fig. 3). The *sim-1 smr1-1* mutant was even more susceptible than *smr1-1*. We were able to rescue the enhanced susceptibility of *smr1-1* and *sim-1 smr1-1* with the *SMR1* genomic construct (Supplemental Fig. S3). In addition, treating *smr1-1* and *sim-1 smr1-1* plants with an SA analog, 300 μM benzo (1, 2, 3) thiadiazol-7-carothioic acid (BTH) that induces similar defense responses as SA (Lawton et al., 1996), rescued the susceptible phenotype of *smr1-1* and *sim-1 smr1-1* (Fig. 3). These results suggest that *SMR1* plays a greater role than *SIM* in defense against *P. syringae* in Arabidopsis and *SMR1* likely acts upstream of the SA pathway.

SMR1 Contributes to SA-Mediated Defense

To further test whether *SMR1* regulates SA-mediated defense, we crossed *sim-1* and *smr1-1* mutants into the *acd6-1* background. *acd6-1* is caused by a point mutation in the *ACD6* gene, encoding an ankyrin repeat protein with a transmembrane domain (Rate et al., 1999; Lu et al., 2003). Although its biochemical function is still unclear, ACD6 was shown as a positive regulator of plant defense. The *acd6-1* mutant is gain-of-function in nature and it shows dwarfism, constitutive defense, high levels of SA, and spontaneous cell death phenotypes. Interestingly, the small size of *acd6-1* was shown largely in an inverse correlation with defense levels of the plant. Thus, the change of *acd6-1* dwarfism induced by second-site mutations could be conveniently used as a visual readout to provide a rapid assessment of the effect of these second-site mutations in some genes on defense responses. *acd6-1* has been successfully used in genetic analyses to reveal epistatic relationships between defense mutants and in a genetic screen to identify, to our knowledge, novel defense genes (Song et al., 2004; Lu et al., 2009; Ng et al., 2011; Wang et al., 2011a, 2011b, 2014a). We found that while *acd6-1 sim-1* resembled *acd6-1* in plant morphology, *acd6-1 smr1-1* and *acd6-1 sim-1 smr1-1* were significantly larger than *acd6-1* plants (Fig. 4, A and B). Associating with increased plant size, *acd6-1 smr1-1* and *acd6-1 sim-1 smr1-1* plants accumulated lower levels of SA and exhibited less cell death, compared with *acd6-1* (Fig. 4, C–E). No significant difference was detected between *acd6-1 smr1-1* and *acd6-1 sim-1 smr1-1* in plant size, SA accumulation,

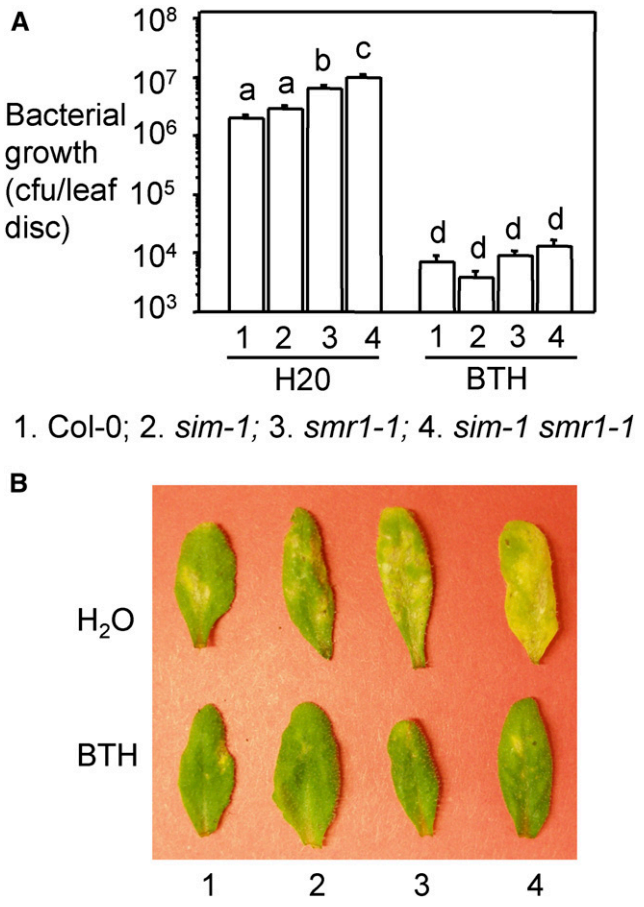

Figure 3. Enhanced disease susceptibility of *smr1-1* and *sim-1 smr1-1* mutants was rescued by BTH treatment. A, Bacterial growth assay. Plants were sprayed with 300 μM BTH or water for 24 h, and the fifth to seventh leaves of the treated plants were infiltrated with the virulent strain PmaDG3 (OD_{600} = 0.0001). Leaf discs were taken 3 d post infection for the measurement of bacterial growth. Statistical analysis was performed with Student's *t* test (StatView 5.0.1). Different letters indicate significant difference among the samples (n = 6; $P < 0.01$). B, Disease symptoms. Pictures of the infected leaves were taken at 4 d post infection. These experiments were repeated twice with similar results.

and cell death. Together, these data indicate that the role of *SMR1* in regulating Arabidopsis defense is through influencing SA accumulation.

Expression Analysis of *SIM* and *SMR1*

Recently Kumar et al. (2015) showed that the multicellular trichome phenotype conferred by *sim-1* could be rescued by a number of *SMR* genes, including *SMR1*, when these genes were artificially expressed under the control of a trichome-specific promoter. Thus, the differential roles of *SIM* and *SMR1* in cell-cycle and defense control are unlikely due to the difference in biochemical function of the gene products, but rather due to transcriptional and/or other posttranscriptional regulations. Indeed we found that expression of *SMR1* was much higher than *SIM* in the leaf tissue (Fig. 5; Supplemental Fig. S4). On the other hand, a microarray study showed that expression of *SIM* is 28-fold enriched in trichomes, compared to leaves with trichomes

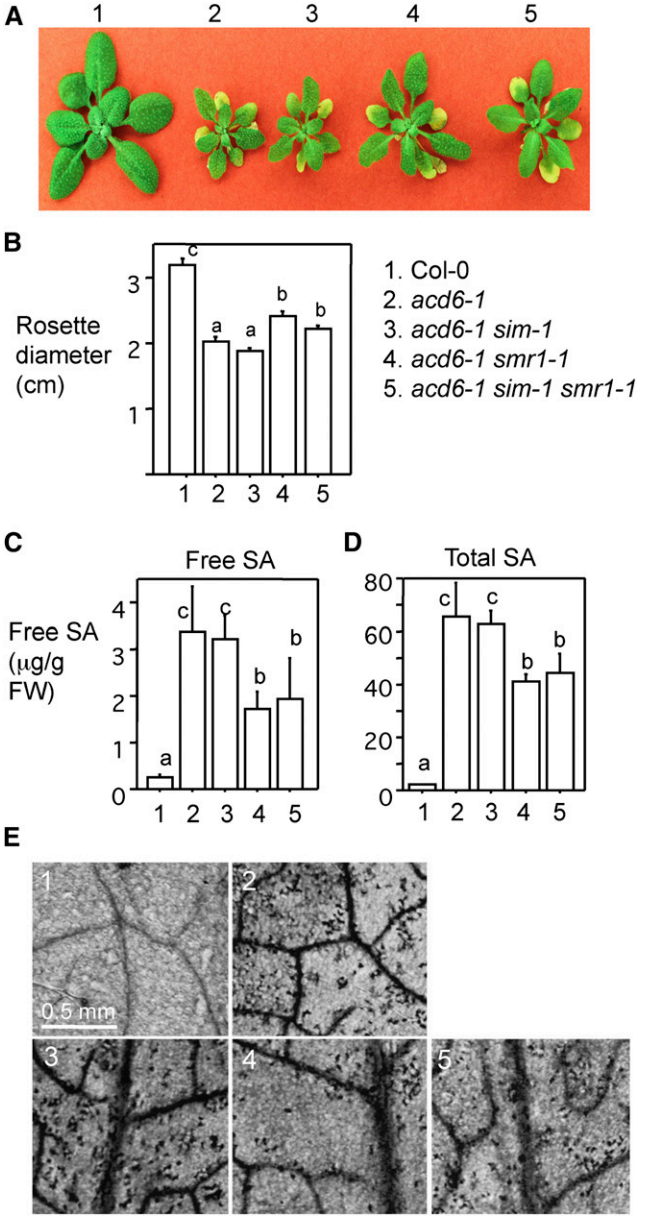

Figure 4. *smr1-1* suppresses dwarfism, SA accumulation, and cell death in *acd6-1*. A, Pictures of 25-d-old plants. Plant genotypes are as follows: 1, Col-0; 2, *acd6-1*; 3, *acd6-1 sim-1*; 4, *acd6-1 smr1-1*; 5, *acd6-1 sim-1 smr1-1*. B, Plant size comparison. Rosette diameters of plants ($n >$ 15) were measured to determine plant size. C, Quantification of free SA level. D, Quantification of total SA level. Free and total SA were extracted from 25-d-old plants and analyzed with an HPLC instrument. E, Images of cell death. The fourth to sixth leaves of each genotype were stained with trypan blue and photographed. Statistical analysis was performed with Student's *t* test (StatView 5.0.1). Different letters indicate significant difference among the samples ($P < 0.05$). These experiments were repeated twice with similar results.

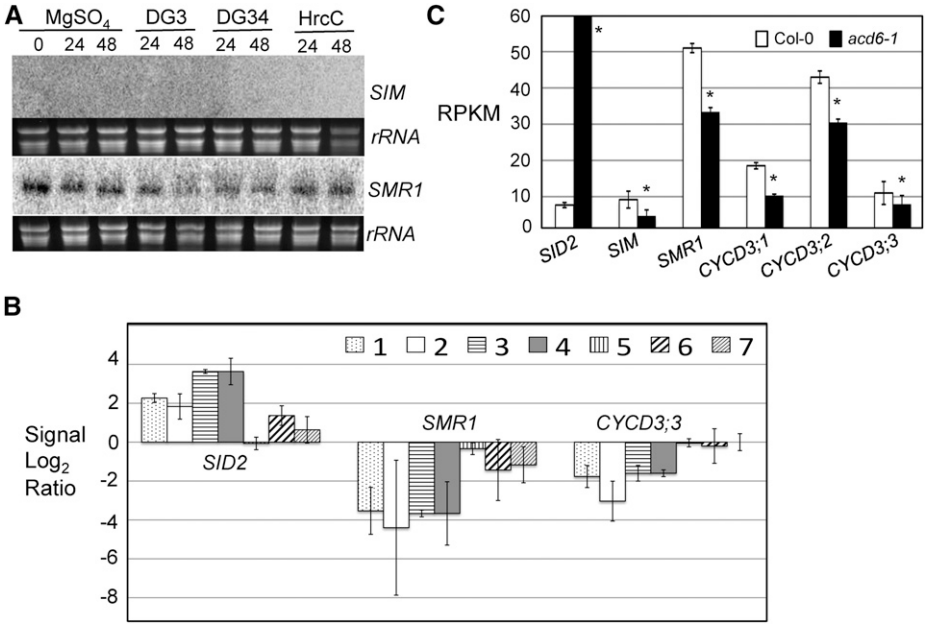

Figure 5. Expression analysis of cell-cycle genes. A, Northern blotting. Total RNA was extracted from 25-d-old plants infected with *P. syringae* strains at the indicated time points. These experiments were repeated twice with similar results. B, Gene expression analysis based on published microarray data. The comparisons are as follows: 1, Pst DC3000 versus mock, 1×10^6 bacteria/mL 24 h post infection (plants were infected with Pst DC3000 at 1×10^6 bacteria/mL or mock-treated and harvested 24 h post infection); 2, Pst DC3000 versus mock, 1×10^8 bacteria/mL 7 h post infection; 3, Pst COR$^-$ versus mock, 1×10^6 bacteria/mL 24 h post infection; 4, Pst COR$^-$ versus mock, 5×10^7 bacteria/mL 10 h post infection; 5, Pst COR$^-$hrpS versus mock, 1×10^6 bacteria/mL 24 h post infection; 6, Pst COR$^-$hrpS versus mock, 5×10^7 bacteria/mL 10 h post infection; and 7, Pst hrpA versus mock, 1×10^8 bacteria/mL 7 h post infection. The signal $\log_2$ ratio for each pathogen infection per gene was average of three samples ± SD. Details for the experiments were described previously (Thilmony et al., 2006). The original values for signal $\log_2$ ratio are listed in Supplemental Table S1 in the article. Only genes whose transcript levels showed 2-fold or more changes are listed in the table. Expression of *SID2* was included as a control. C, Quantification of gene expression based on RNAseq. Total RNA extracted from Col-0 and *acd6-1* was used for making cDNA libraries, followed by high-throughput sequencing using Illumina HiSEquation 2000 platform. RPKM were average of three samples ± SD. The RPKM value for *SID2* is 174 and is over the scale limit, which was set to show the lower expression of other genes. Asterisks indicate that expression of a gene in *acd6-1* is significantly different from its expression in Col-0 ($P < 0.05$).

removed. *SMR1* did not show up as a significantly trichome-enriched gene in this study (Jakoby et al., 2008). In addition, another microarray study showed that *SIM* expression was found to be highest in first leaves at 9 d post germination, when leaves are about 200 microns long and trichome initiation is the greatest, and decline thereafter, while *SMR1* expression increases approximately 5-fold from 9 to 22 d post germination (Beemster et al., 2005; Supplemental Fig. S4A). Thus, spatial and temporal expression patterns of *SIM* and *SMR1* are important in determining the biological processes regulated by these genes.

Consistent with the major role of *SMR1* in defense control, we found that expression of *SMR1* was suppressed by infection of both virulent and avirulent *P. syringae* strains, PmaDG3 and PmaDG34 avrRpm1, respectively (Fig. 5A; Supplemental Fig. S4B). This observation is further supported by *in silico* analysis of a microarray dataset involving RNA samples isolated from Arabidopsis leaves infected with different *P. syringae* strains (Thilmony et al., 2006). *P. syringae* pv tomato DC3000 (Pst DC3000) is a virulent strain producing the phytotoxin coronatine (COR), while Pst COR$^-$ lacks COR production (Mittal and Davis, 1995; Bender et al., 1999). *HrpS* is a regulatory gene of *P. syringae* that could affect the formation of type 3 secretion system and COR production (Roine et al., 1997). *HrpA* encodes the main structural protein for the of type 3 secretion system pilus (Wei et al., 2000). Both *P. syringae hrpS* and *hrpA* mutant strains are unable to deliver bacterial effector proteins into the host cells, thus only inducing PAMPs-triggered immunity, but not effector triggered immunity in the host. Like PmaDG3, Pst DC3000 infection at both doses (1×10^6 bacteria/mL and 1×10^8 bacteria/mL) suppressed expression of *SMR1* (Fig. 5B). The lack of COR production by the Pst DC3000 did not affect such suppression, suggesting Pst DC3000-induced suppression of *SMR1* is COR independent. Infection with *Pst* COR$^-$ *hrpS* and *Pst hrpA* also suppressed *SMR1* expression, albeit at a lower level. These results suggest that bacterial effector(s) have stronger influence than PAMP molecules in suppressing *SMR1* expression.

Since our data showed that *SMR1* affects SA-mediated defense (Fig. 4), we sought to further examine whether expression of *SMR1* is reciprocally affected by SA. We first analyzed the microarray dataset that has triplicate samples harvested two hours after mock or 1 mM SA treatment of Arabidopsis leaves (Thibaud-Nissen et al., 2006; data accessible at NCBI GEO database, accession no. GSE3984). We found that there was a small suppression of *SMR1* expression in the SA-treated sample (7.89 ± 0.27 counts), compared with the control (9.72 ± 0.60 counts), suggesting that SA negatively regulates *SMR1* expression. To further validate SA suppression of *SMR1* expression, we performed a high-throughput RNAseq experiment, using the SA-hyperaccumulating mutant *acd6-1* and Col-0 as a control. Analysis of the RNAseq data revealed that expression of *SMR1* was significantly lower in *acd6-1* than in Col-0 (Fig. 5C). *SIM* expression was low in Col-0 and was further reduced in *acd6-1*. On the other hand, expression of *SID2* (a positive control) was highly induced in *acd6-1*. Together these data suggest that while *SIM* and *SMR1* genes can positively affect defense, defense activation can also feedback to inhibit expression of these genes. Such a negative feedback loop between the cell cycle and immune responses of Arabidopsis could be important for a tight control of these two biological processes during plant growth and development and biotic stress conditions.

SA Signaling Affects Cell-Cycle Progression

Besides *SMR1*, two other cell-cycle regulators, *CPR5* and the noncanonical *E2F* gene *DEL1*, were shown to affect SA-mediated defense (Chandran et al., 2014; Wang et al., 2014b). These results prompted us to ask whether SA could affect cell-cycle progression as part of downstream signaling of some cell-cycle genes. To test this, we analyzed cell ploidy in the major SA biosynthetic mutant *sid2-1* (Wildermuth et al., 2001; Ng et al., 2011) and the SA signaling mutant *npr1-1* (Dong, 2004; Lu, 2009; Fu and Dong, 2013). Our results show that both *sid2-1* and *npr1-1* had significantly increased numbers of 4C cells but reduced numbers of 8C and 16C cells (Fig. 6). The ploidy indices of *sid2-1* and *npr1-1* were 2.6 ± 0.2 and 2.7 ± 0.1, respectively, significantly lower than that of Col-0 (3.0 ± 0.2). Thus, these data suggest that reducing SA levels and blocking SA signaling could lead to reduced endoreplication in Arabidopsis. However, when we treated Col-0 plants with the SA activator, 300 μM BTH, we did not detect any changes in cell ploidy pattern 24 and 72 h post BTH treatment (Supplemental Fig. S5). Longer BTH treatment up to 12 d with plants being BTH-sprayed every 4 d did not reveal significant difference in the cell ploidy pattern (data not shown). Thus, these results suggest that SA is necessary, but not sufficient, to affect cell-cycle progression.

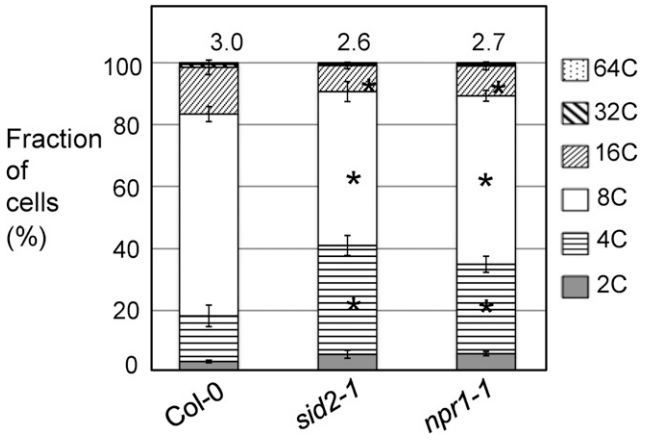

Figure 6. Altered cell ploidy in *sid2-1* and *npr1-1* mutants. The fourth to sixth leaves of 25-d-old plants were collected for nuclei isolation, followed by flow cytometry analysis. Data represent the average of five experiments ± SE of mean. Statistical analysis was performed with Mann-Whitney test (http://vassarstats.net/). Asterisks indicate significant difference between Col-0 and *sid2-1* or *npr1-1* in the same ploidy group ($P < 0.05$). Ploidy indices were shown above the bars.

The *CYCD3* Genes Contribute to Defense

Our results showed that decreased cell ploidy in *smr1-1*, *sim-1 smr1-1*, and the SA mutants is associated with increased disease susceptibility to *P. syringae*. To further test how an increase in cell ploidy could affect Arabidopsis defense, we examined defense responses of a triple mutant with three *CYCD3* genes disrupted, *cycd3;1,2,3*, which was shown to have a higher ploidy level than wild type (Dewitte et al., 2007). We chose to work on this mutant is also because CYCD3 proteins are potential targets of CKIs and are known to play a key role in transitions from G1-S and from cell proliferation to endoreplication (Riou-Khamlichi et al., 1999; Dewitte et al., 2003; Churchman et al., 2006; Van Leene et al., 2010).

While *SIM* and *SMR1* are positive regulators of endoreplication, the *CYCD3* genes are considered negative regulators. Interestingly, when infected with PmaDG3, the *cycd3;1,2,3* mutant showed increased disease susceptibility associated with more severe disease symptoms (Fig. 7, A and B). This susceptibility of *cycd3;1,2,3* could be rescued with exogenous BTH treatment. However, *cycd3;1,2,3* was not susceptible to the avirulent strains PmaDG6 (expressing the avirulence effector *avrRpt2*) and PmaDG34 (expressing the avirulence effector *avrRpm1*; Hamdoun et al., 2013; Fig. 7C). Neither did we detect a compromised hypersensitive response in this mutant to the infection of the two avirulent strains (Supplemental Fig. S6). These results suggest that *CYCD3;1,2,3* genes are involved in affecting basal defense in Arabidopsis.

Similar to *SMR1*, expression of a *CYCD3* gene, *CYCD3;3*, was suppressed by Pst DC3000 infection independent of bacterial COR production (Fig. 5B). In addition, the transcript levels of *CYCD3;1*, *CYCD3;2*,

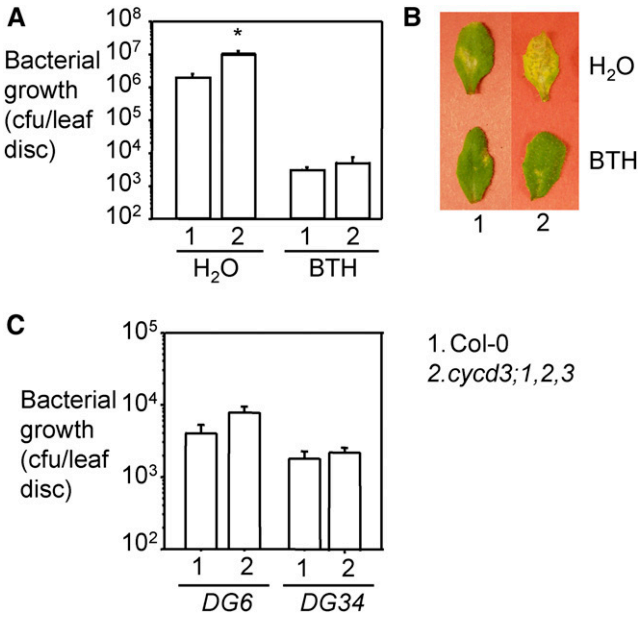

Figure 7. Enhanced disease susceptibility of the *cycd3;1,2,3* mutant was rescued by BTH treatment. A, Bacterial growth assay with PmaDG3. Plants were sprayed with 300 μM BTH or water for 24 h, and the fifth to seventh leaves of the treated plants were infiltrated with the virulent strain PmaDG3 (10^5 bacteria/mL). Leaf discs were taken 3 d post infection for the measurement of bacterial growth. Statistical analysis was performed with Student's *t* test (StatView 5.0.1). The asterisk indicates significant difference between Col-0 and *cycd3;1,2,3* with water treatment ($n = 6$; $P < 0.05$). B, Disease symptoms. Pictures of the infected leaves were taken at 4 d post infection. C, Bacterial growth assay with PmaDG6 or PmaDG34 infection. These experiments were repeated twice with similar results.

and *CYCD3;3* were significantly lower in the constitutive defense mutant *acd6-1* than in Col-0 (Fig. 5C). These results further support a negative feedback regulatory mechanism in controlling expression of some cell-cycle genes and immune activation in Arabidopsis.

DISCUSSION

In this report we presented data to show that two homologous *CKI* genes, *SIM* and *SMR1*, play differential roles in regulating cell ploidy and disease resistance to the bacterial pathogen *P. syringae*. We provide a mechanistic explanation of such differential roles of the two genes, which is mainly due to transcriptional regulation of these two genes during development, in different tissue, and in response to pathogens. Our data further reveal a negative feedback regulation between *SMR1*-mediated defense and cell-cycle control. While supporting that cell-cycle progression is intimately related to plant defense, our data also raise an interesting question regarding relationship between the ploidy level and defense.

The *SIM* and *SMR1* genes belong to a family of plant-specific *CKI*s. Although sharing over 60% identity in their encoded amino acids, *SIM* and *SMR1* show both distinct and overlapping functions in cell-cycle and defense regulation. We presented in this report several pieces of evidence to support this notion: (1) *SIM* plays a stronger role in affecting endoreplication in trichomes, while *SMR1* affects leaf cell ploidy more strongly; (2) the *smr1-1* mutant, but not *sim-1*, was more susceptible to *P. syringae* infection and showed suppression of dwarfism, cell death, and SA accumulation in *acd6-1*; (3) a *SMR1* genomic fragment rescued severe multicellular trichome phenotype of *sim-1 smr1-1* but did not rescue trichome defects of *sim-1*; and (4) knockout of both genes resulted in more severe ploidy defects in trichomes and leaf cells and further enhanced susceptibility to *P. syringae* infection. Since SIM and SMR1 were shown to bear similar biochemical activities when being overexpressed in trichome cells (Kumar et al., 2015), the differential functions displayed by the two homologous genes are likely due to their differential expression patterns. Indeed, we found that *SIM* was enriched in the trichome while *SMR1* was enriched in nontrichome leaf cells. In addition, expression of *SMR1* was suppressed by *P. syringae* infection and in the constitutive defense mutant *acd6-1*, consistent with a stronger role of *SMR1* in defense. Other cell-cycle genes, such as *SIM* and *CYCD3*, were also suppressed during defense activation (Fig. 5). The initial high levels of *SMR1* (possibly also other cell-cycle genes) could preemptively guard against pathogens. Suppression of these genes after defense activation could reflect an adjustment in balancing development and the costly defense responses in Arabidopsis.

Besides the *SIM* and *SMR1* genes, other *SMR* family members have not been demonstrated to have roles in plant defense, although several studies have implicated some *SMR* genes in stress responses. For instance, expression of several Arabidopsis *SMR* genes, as well as a rice homolog of *SIM*, is altered in response to biotic and/or abiotic stress conditions (Peres et al., 2007; Chandran et al., 2009; Yi et al., 2014). Two *SIM* homologs, *SMR5* and *SMR7*, were shown to be important for plant response to DNA damage (Yi et al., 2014). In addition, several *SMR* genes, including *SMR1*, *-2*, *-3*, *-4*, *-5*, *-7*, *-11*, and *-13*, complemented the multicellular trichome defects of *sim-1*, when these genes were expressed in the trichome using a trichome-specific promoter (Kumar et al., 2015). Thus, SMR proteins share high conservation as SIM, and it is possible that additional *SMR* family members are important for plant innate immunity. It would be interesting to identify and characterize such *SMR* genes and determine their functions in cell-cycle and defense control.

SA is a small signaling molecule important for biotic responses of plants. Our data presented here and in earlier studies (Vanacker et al., 2001; Hamdoun et al., 2013) suggest SA-mediated pathway is an integral part of cell-cycle signaling. The lack of SA biosynthesis and signaling in *sid2-1* and *npr1-1*, respectively, had reduced overall ploidy pattern of leaf cells when assayed by flow cytometry (Fig. 6). Interestingly, our previous

study using nuclear quantification of mesophyll cells from fixed sections near the middle vein of *npr1-1* showed increased ploidy, suggesting a possibility that *npr1-1* leaves harbor cells with different ploidy. Both *sid2-1* and *npr1-1* mutants were also compromised in *P. syringae*-induced large cell growth (Hamdoun et al., 2013). Since activation of SA signaling with exogenous application of an SA analog did not change cell ploidy and the formation of tumor-like growth in the leaf (Supplemental Fig. S5; Hamdoun et al., 2013), it is possible that SA signaling requires the activation of additional pathways to regulate cell-cycle progression.

Our data show that *SMR1* acts partly through SA in defense regulation. We found that the susceptibility of *smr1-1* was rescued by exogenous activation of SA signaling and *smr1-1* suppressed SA accumulation in *acd6-1* (Figs. 3 and 4). Consistent with our data, Wang et al. showed that the *sim smr1* mutant suppressed high SA levels of the *cpr5* mutant and exhibited a delay in SA accumulation with the infection of the avirulent *P. syringae* strain expressing *avrRpt2* (Wang et al., 2014b). *CPR5* encodes a nuclear membrane protein that interacts with SIM and SMR1. Mutations in the *CPR5* gene confer constitutive defense and higher SA accumulation (Bowling et al., 1997; Clarke et al., 2001; Jirage et al., 2001; Brininstool et al., 2008). Thus, *SIM/SMR1* and *CPR5* are positive and negative regulators of the SA pathway, respectively.

On the other hand, like *SIM* and *SMR1*, studies suggest that *CPR5* promotes endoreplication, possibly through the canonical cell-cycle signaling pathway involving *RBR-E2F* (Kirik et al., 2001; Wang et al., 2014b). The SA pathway influenced by *CPR5-SIM/SMR1* could be convergent with the canonical *RBR-E2F* pathway; for instance, *CPR5-SIM/SMR1* act through *RBR-E2F* signaling to regulate SA accumulation. However, it is currently unknown if RBR-E2F signaling could affect SA-mediated defense besides its role in cell-cycle control. Another possible downstream cell-cycle component of *CPR5-SIM/SMR1* could be the non-canonical *E2F* gene, *DEL1*, whose protein product was shown to bind directly to the main SA regulatory gene *EDS5* (Nawrath and Métraux, 1999; Nawrath et al., 2002; Chandran et al., 2014). It is also possible that *CPR5-SIM/SMR1* may act through a pathway independent of cell-cycle signaling in regulating SA-mediated defense.

One interesting observation that we had during the course of the study is that there appears to be no direct correlation between the ploidy level and defense. *sim-1*, *smr1-1*, *sid2-1*, and *npr1-1* mutants had lower cell ploidy and reduced disease resistance, suggesting a positive association of the two processes. However, results with the *cycd3;1,2,3* mutant that has increased cell ploidy and decreased resistance to *P. syringae* would challenge such a statement. Indeed, analysis of additional cell-cycle mutants recently reported to have defense defects showed no direct association between the ploidy level and defense response. For instance, *cpr5-1* had a lower ploidy but was highly resistant (Bowling et al., 1997; Clarke et al., 2001; Jirage et al., 2001; Kirik et al., 2001). The loss-of-function and gain-of-function mutations in the *Omission of the Second Division 1* gene, encoding a negative regulator of anaphase-promoting complex/cyclosome, resulted in higher and lower cell ploidy, respectively. Despite of their opposite effects on the cell cycle, both types of plants were resistant to *P. syringae* infection (Bao et al., 2013; Bao and Hua, 2014). Thus, it is not the level of cell ploidy that affects host defense. Rather, it is the disruption of the normal cell-cycle progression that results in altered host response to pathogen invasion.

How does one explain such a dissociation of ploidy and defense levels? One possibility is that there are different modes of action used by cell-cycle genes in cell-cycle and defense control. For instance, CPR5, SIM, and SMR1 are positive regulators to promote endoreplication, and these proteins may execute this function by physical interaction with each other (Wang et al., 2014b). The increased phosphorylation in the RBR protein in the *sim smr1* and *cpr5* mutants is consistent with the roles of *CPR5-SIM/SMR1* proteins in inhibiting activities of CYC/CDK complexes and promoting endoreplication. On the other hand, *sim smr1* and *cpr5* mutants exhibited opposing defense phenotypes, suggesting that *CPR5* antagonizes *SMR1* (and/or *SIM*) in defense control. In this regard, it is possible that *SMR1* (and/or *SIM*) act downstream of *CPR5* in regulating disease resistance based on the suppression of defense phenotypes of a *cpr5* mutant by *sim* and *smr1* mutations (Wang et al., 2014b). In *CPR5-SIM/SMR*-mediated defense pathway, there might be additional genes that could differentially interact with *CPR5* and *SIM/SMR1* to positively or negatively regulate plant defense.

SIM and SMR1 are CKIs that target CYC/CDK complexes to promote endoreplication. One of such targets could be CYCD3 proteins. Indeed, SIM was shown to interact with the CYCD3;2 protein when both proteins transiently expressed in Arabidopsis leaf cells (Churchman et al., 2006). SIM was also shown to interact with CDKA;1, which is activated by CYCD3;1, and inhibits activities of CYCD3;1/CDKA;1 and several other CYC/CDK complexes (Kumar et al., 2015). The decreased ploidy in *sim-1 smr1-1* and increased ploidy in *cycd3;1,2,3* is consistent with SIM and SMR1 being inhibitor of CYCD3/CDKA complexes and that these two genes act in opposing ways to regulate endoreplication. However, *SIM*, *SMR1*, and *CYCD3* genes appear to be positive regulators of plant defense because mutations in the corresponding genes conferred enhanced disease susceptibility to *P. syringae.* In this case, SIM and SMR1 could positively affect *CYCD3* function in defense control. Such positive and negative roles of SIM and SMR1 have already been reported for other cell-cycle genes (Sherr and Roberts, 1999). Alternatively, *SIM/SMR1* could act in separate pathways from the *CYCD3* genes to affect the cell cycle and defense in Arabidopsis. To support this latter speculation,

CYCD3;2 was found to copurify with two other SMR family members, SMR4 and SMR6 (Van Leene et al., 2010), implicating that CYCD3;2 could interact with different partners in executing its functions in control of cell-cycle progression and defense.

The molecular mechanism underlying the crosstalk between cell-cycle progression and innate immune control is far from being understood. It is critically important to elucidate the mechanisms of action of core cell-cycle genes in defense control. Further identification and characterization of additional genes that are involved in cell-cycle and defense control should also help to gain a better understanding of processes involved in cell-cycle control and plant defense.

MATERIALS AND METHODS

Plant Materials

All plants used in this report were in Col-0 background and were grown in growth chambers with a 12 h light/12 h dark cycle, light intensity at 200 μmol m^{-2} s^{-1}, 60% humidity, and 22°C. The mutants *acd6-1*, *sim-1*, *cycd3;1,2,3*, *npr1-1*, and *sid2-1* were previously described (Walker et al., 2000; Lu et al., 2003; Churchman et al., 2006; Dewitte et al., 2007; Wang et al., 2011a). The *smr1-1* (SALK_33950) was obtained from the Arabidopsis Biological Research Center. *sim-1 smr1-1*, *acd6-1 sim-1*, *acd6-1 smr1-1*, and *acd6-1 sim-1 smr1-1* mutants were generated by genetic crosses. All mutations were confirmed by genotyping with specific primers (Supplemental Table S1; Lu et al., 2003).

Generate Transgenic Plants

A 2367 bp *SMR1* genomic fragment including 1424 bp promoter and 637 bp 3′ downstream of the *SMR1* gene was amplified by PCR with the primers genomicSMR1-For1 and genomicSMR1-R1 (Supplemental Table S1) and cloned into the binary vector pGreen0229 (Hellens et al., 2000) for transformation of *smr1-1* or *sim-1 smr1-1* plants. At least 10 independent transformants from each background were obtained for further selection of homozygous transgenic plants, followed by analyses for gene expression and disease resistance to *P. syringae*.

Pseudomonas syringae Infection

The virulent strain *P. syringae* pv *maculicola* ES4326 DG3 (PmaDG3) and the avirulent strains PmaDG6 (expressing the avirulence effector *avrRpt2*) and PmaDG34 (expressing the avirulence effector *avrRpm1*; Hamdoun et al., 2013) were used to infect plants. Bacterial culture, preparation, inoculation by infiltration, and bacterial growth assay were conducted as described (Lu et al., 2013). For BTH treatment, plants were sprayed with 300 μM BTH (a kind gift from Robert Dietrich [Syngenta]) or water for 24 h before being infected with PmaDG3. For the hypersensitive response, a half area of a leaf at the fourth to sixth position of each genotype was infiltrated with a *P. syringae* strain (OD_{600} = 0.1) and examined for leaf wilting and collapse 18 h post infection. At least 15 leaves from at least six plants were used for each treatment group.

RNA Analysis

For *P. syringae*-challenged plants, the infected leaves were harvested for RNA extraction. For noninfected plants, all leaves were used for RNA preparation. The *SIM* and *SMR1* probes were made by PCR with gene specific primers (Supplemental Table S1) and labeled with [^{32}P]dCTP, using a corresponding antisense primer.

For quantitative reverse transcription PCR, 2 μg of total RNA was reversed-transcribed into cDNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) according to the manufacturer's instructions. cDNA was diluted 20 times for subsequent quantitative PCR reactions, which were set up using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific). Three milliliters of diluted cDNA template was used in a 20 mL PCR reaction. Primers for detection of transcripts of *SIM*, *SMR1*, and *PP2AA3* (as a control) are listed in Supplemental Table S1. The quantitative PCR reactions were run in a CFX96 Touch Real-Time PCR System (Biorad) with one step at 95°C for 5 min, 40 cycles at 95°C for 10 s, 52°C for 15 s, and 72°C for 15 s. PCR products were checked using melting curve analysis to ensure amplification of a single product and the absence of primer-dimers. For relative gene expression at different time points, the $2^{-\Delta CT}$ method was applied as described (Schmittgen and Livak, 2008), using the cycle threshold values of *PP2AA3* for normalization.

RNAseq Library Preparation, Sequencing, and Analysis

Total RNA was extracted from 25-d-old Col-0 and *acd6-1* plants using Qiagen RNeasy plant mini kit (catalog no. 74903). On-column digestion was performed using Qiagen RNase free DNase kit (catalog no. 79254) to remove genomic DNA contamination. Each sample had three biological replicates, and 0.5 μg RNA per replicate was used to generate cDNA libraries using Illumina TruSeq RNA sample preparation kit (catalog no. RS-122-2001) for deep sequencing in Genomics Resources Core Facility at Weill Cornell Medical College. The samples were multiplexed and sequenced with the standard run of 51-cycle and single-read. At least 150 million reads per lane were obtained.

The raw reads in FASTQ format were imported into CLC Genomics Workbench 8 (Qiagen) and mapped to the TAIR 10 Arabidopsis reference genome annotated with genes and transcripts. Gene Expression files of each sample were generated based on the total number of reads mapped to individual genes. Gene Expression files from different samples were compared in order to identify differentially expressed genes using the following parameters: the absolute value of fold change is > 2; the P value of a false discovery rate is < 0.05, and the value of reads per kilobase of exon per million reads mapped (RPKM) is > 0.3. For the comparison of expression of individual genes between different samples, the original RPKM was used. Statistic analysis was performed using ANOVA post hoc Fisher's PLSD test.

SA Measurement

Free and total (glucosylated) SA were extracted from plants and quantified with an HPLC instrument as previously described (Ng et al., 2011; Wang et al., 2011a).

Cell Death Staining

The fourth to sixth leaves of 25-d-old plants were collected, boiled in lactophenol containing 0.01% trypan blue for 2 min, cleared off by boiling in alcoholic lactophenol, and rinsed with 50% ethanol. The stained leaves were visualized with a dissecting microscope (Leica M80, Leica Microsystems) and photographed with a CCD camera (Leica IC80 HD) connected to the microscope. At least four leaves from four plants of each treatment were stained and examined for cell death.

Epidermal Cell Morphology

The fourth to sixth leaves of 25-d-old plants were harvested, incubated with ethanol to clear off chlorophyll, and examined for epidermal cell morphology. At least three leaves from three plants of each genotype were photographed with a CCD camera (Cool Snap HQ^2, Photometrics) connected to a dissecting microscope (Leica M205 FA, Leica Microsystems).

Scanning Electron Microscopy

First leaves of 2-week-old Arabidopsis plants were mounted on the specimen stubs using double-stick tape and were observed under high vacuum mode at 5.0 kV in a JEOL JSM 6610LV scanning electron microscope, working quickly to avoid drying and damage from the beam.

Quantification of Trichome Initiation Site

Trichome nuclei staining with 4′,6-diamidino-2-phenylindole was performed as described (Walker et al., 2000). DAPI-stained nuclei per trichome initiation site were counted at either 200× or 400× magnification using a Leica DM RXA2 microscope, and images were captured with a SensiCamQE 12-bit cooled CCD camera.

Flow Cytometry Analysis

The fourth to sixth leaves of 25-d-old plants were harvested and coarsely chopped with a single-edge razor blade into a mince in "Aru" buffer containing 97.5% $MgSO_4$ (0.246% $MgSO_4$ $7H_2O$, 0.37% KCl, and 0.12% HEPES), 0.1% DTT, and 2.5% Triton X-100 (Arumuganathan and Earle, 1991) in order to release nuclei. At least 10 leaves were used for each sample. The mince was passed through a nylon mesh with pore size 40 μm to collect nuclei. Nuclei were stained with propidium iodide at the final concentration 30 μg/mL and were subjected to flow analysis with a CyAn ADP flow cytometer (Beckman Coulter). Data were analyzed using Summit software (v4.3.02). Propidium-iodide-stained nuclei were detected using a blue laser (emission 488 nm and excitation 613/620 nm) and 605 voltages power output. At least 20,000 events were recorded at the flow rate of 150 events/second for each sample. Triplicate samples per treatment and/or per genotype were included. Ploidy index was calculated as follows: ploidy index = (% 2C nuclei × 1) + (% 4C nuclei × 2) + (% 8C nuclei × 3) + (% 16C nuclei × 4) + (% 32C nuclei × 5) + (% 64C nuclei × 6).

Accession Numbers

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers At5g04470 for SIM and At3g10525 for SMR1.

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. Trichome morphology.

Supplemental Figure S2. Rescue trichome phenotype of *sim-1 smr1-1* to that of *sim-1* by a *SMR1* genomic fragment.

Supplemental Figure S3. Complementation of enhanced disease susceptibility of *sim-1 smr1-1* and *smr1-1* by a *SMR1* genomic fragment.

Supplemental Figure S4. Expression analysis of *SIM* and *SMR1*.

Supplemental Figure S5. BTH treatment does not affect cell ploidy in Arabidopsis.

Supplemental Figure S6. The *cycd3;1,2,3* mutant is not compromised to the hypersensitive response induced by *PmaDG6* or *PmaDG34*.

Supplemental Table S1. Primers used in this paper.

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