Arabidopsis BRCA2 and RAD51 proteins are specifically involved in defense gene transcription during plant immune responses

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Systemic acquired resistance (SAR) is a plant immune response associated with both transcriptional reprogramming and increased homologous DNA recombination (HR). SNI1 is a negative regulator of SAR and HR, as indicated by the increased basal expression of defense genes and HR in *sni1*. We found that the *sni1* phenotypes are rescued by mutations in *BREAST CANCER 2* (*BRCA2*). In humans, BRCA2 is a mediator of RAD51 in pairing of homologous DNA. Mutations in *BRCA2* cause predisposition to breast/ovarian cancers; however, the role of the BRCA2–RAD51 complex in transcriptional regulation remains unclear. In *Arabidopsis*, both *brca2* and *rad51* were found to be hypersusceptible not only to genotoxic substances, but also to pathogen infections. A whole-genome microarray analysis showed that downstream of NPR1, BRCA2A is a major regulator of defense-related gene transcription. ChIP demonstrated that RAD51 is specifically recruited to the promoters of defense genes during SAR. This recruitment is dependent on the SAR signal salicylic acid (SA) and on the function of BRCA2. This study provides the molecular evidence showing that the BRCA2–RAD51 complex, known for its function in HR, also plays a direct and specific role in transcription regulation during plant immune responses.

suppressor of sni1 3 | tiling array-based cloning | plant fertility | transcription-associated DNA damage | chromatin remodeling

Systemic acquired resistance (SAR) is an inducible broad-spectrum immune response in plants (1). The onset of SAR involves transcriptional reprogramming of as many as 10% of the genes in a plant genome (2), and thus must be tightly regulated to minimize pleiotropic effects on normal plant growth and development. Genetic screens have identified NPR1 (NONEXPRESSOR OF PR1 GENES 1) and SNI1 (SUPPRESSOR OF NPR1-1 INDUCIBLE 1) as crucial positive and negative regulators of SAR, respectively. The accumulation, nuclear translocation, and turnover of the transcription cofactor NPR1 are triggered by the SAR signal salicylic acid (SA), leading to the production of antimicrobial pathogenesis-related (PR) proteins (3–5). In the *npr1* mutant, *PR* gene induction and SAR are completely blocked. The transcriptional inducibility of *PR* genes, as well as disease resistance, are restored in the *npr1 sni1* double mutant, suggesting that SNI1 is a transcription repressor functioning downstream of NPR1 (6). Consistent with this hypothesis, the *sni1* single mutant exhibits elevated basal expression of *PR* genes as well as retarded plant growth. Whole-genome microarray analysis has shown that >95% of the genes with elevated expression in *sni1* are SAR-related and NPR1-dependent (7).

Besides defense gene expression, the *sni1* mutant also displays an increase in homologous DNA recombination (HR). A possible direct link between SAR-mediated gene expression and HR is further supported by the finding that *ssn1* (suppressor of *sni1* 1) is a mutant of *RAD51D* (8). The *rad51d* mutation not only abolished the transcriptional inducibility of *PR* genes observed in the *npr1 sni1* mutant, but also decreased the frequency of HR in the *sni1* mutant. The role of RAD51D, a paralog of the RAD51 recombinase, in catalyzing DNA pairing and strand exchange during HR has been studied in mammals (9, 10). Defects in the *RAD51* family genes (including *RAD51D*) lead to genomic instability and, in mammals, tumor formation (11). Although little is known about the role of this gene family in transcription regulation, studies in bacteria, yeast, and mammals have shown that actively transcribed genes are more vulnerable to mutations and recombination, known as transcription-associated mutations and transcription-associated recombination (12). Understanding the connection between plant immune responses and genome stability has overarching significance in our study of plant–microbe interactions and coevolution. The discovery of the genetic interaction between *sni1* and *rad51d* in SAR as well as HR suggests that SNI1–RAD51D might be a molecular link between these two fundamental stress responses (8, 13).

To gain insight into the molecular interactions between responses to pathogen infection and responses to genome stress, we characterized another suppressor of *sni1* mutant, *ssn3,* and found that it encodes BRCA2A (BREAST CANCER 2A). BRCA2 is known to complex with RAD51 and regulate the binding of RAD51 to DNA during HR and DNA repair (14, 15). In this study, we show that downstream of NPR1, BRCA2A is a major regulator of plant defense gene transcription. Upon SA treatment, BRCA2A is required for the recruitment of RAD51 to the *PR* gene promoters, suggesting that the BRCA2A–RAD51 complex might play a direct role in the chromatin remodeling required for transcription as well as in the repair of transcription-associated DNA damage.

Results

Identification of the *ssn3* Mutant. To gain insight into the molecular link between plant immunity and HR, we characterized the *ssn3* mutant, which was identified in a population generated by fast neutron mutagenesis in the *sni1* mutant background (8). Through genetic complementation analysis, we found five alleles of *ssn3*, initially designated 6C-1, 9F-1, 13G-1, 13F-1, and 21B-1. In the *sni1 ssn3* double mutant, the pleiotropic morphological phenotypes of *sni1*, including dwarfism and distorted leaves, were fully suppressed in 4-wk-old mature plants (Fig. 1*A*). We also examined the effect of the *ssn3* mutation on the transcriptional inducibility of *PR* genes using the SA-responsive *BGL2::GUS* transgene as a reporter (3). When treated with SA or its functional analog 2, 6-dichloronicotinic acid (INA), the induction of *BGL2::GUS* was abolished in the *npr1* mutant, but was restored by the *sni1* mutation in the *npr1 sni1* mutant (Fig. 1*B*).

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Data deposition: The microarray data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE23617).

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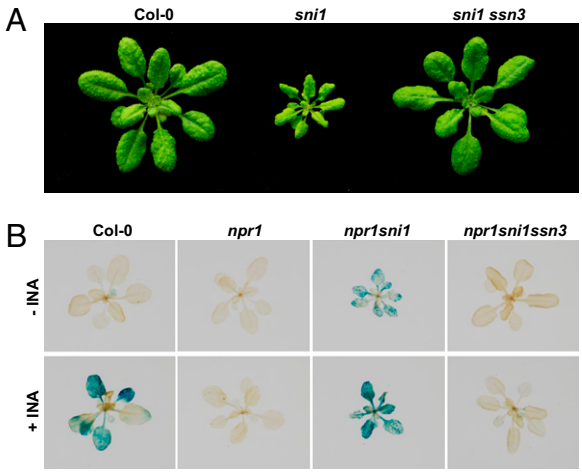

Fig. 1. The *ssn3* mutant is a suppressor of *sni1*. (*A*) Morphology of WT (Col-0), *sni1*, and *sni1 ssn3* plants after 4 wk of growth in soil. (*B*) Expression of the SA-responsive reporter *BGL2::GUS* was detected in 4-wk-old WT, *npr1*, *npr1 sni1*, and *npr1 sni1 ssn3* plants 16 h after spraying with water (–INA) or 0.5 mM INA (+INA).

In the *npr1 sni1 ssn3* triple mutant, the expression of *BGL2::GUS* was once again lost due to the *ssn3* mutation, confirming that *ssn3* is a suppressor of *sni1* and indicating that SSN3 is required for the SA-dependent but NPR1-independent *PR* gene induction observed in *sni1*.

Cloning of *SSN3* Using a Tiling Array–Based Approach. Given the difficulties that we encountered when fine-mapping *ssn3*, we sought a different method for cloning the *SSN3* gene. Because the five different *ssn3* mutant alleles were generated using fast neutron bombardment, which is known to produce genomic deletions (16), we used the GeneChip *Arabidopsis* Tiling 1.0R Array (Affymetrix) to detect possible deletions in the *SSN3* gene. For this, three *sni1 ssn3* alleles (9F-1, 13F-1, and 13G-1) were backcrossed with the *sni1* parent, and eight of the F2 progeny showing the *sni1 ssn3* phenotype were pooled for each allele. Genomic DNA was extracted from the progeny pools, labeled, and used to hybridize with the tiling array. Deletions were expected to result in reduced or missing hybridization signals in mutant samples compared with WT. The chip data were then subject to *P* value interval analysis using Affymetrix Tiling Array Software. As shown in Fig. 2*A*, in the 9F-1 allele, a deletion (outlined in red) began at the very top of chromosome IV and extended to the 3′ region of the *BRCA2A* gene. A large deletion (>10 kb) present in the 9F-1 allele as a PCR product from the F2 and R primers (Table S1) was detected in 9F-1, but not in WT, due to the long distance between the primers (Fig. 2*B*). Subsequent sequencing analysis identified mutations in all other *ssn3* alleles. The 6C-1 allele has a rearrangement near the start codon of *BRCA2A* (between GGC GA and *T GGT* in the nucleotide residues GGC GA*T GGT*, coding for Gly_{12}, Asp_{13}, and Gly_{14}). The 13F-1 allele carries two substitutions in the middle of *BRCA2A*; the nucleotide residues TCC AGA AAG, coding for Ser_{570}, Arg_{571}, and Lys_{572}, were substituted by TCT AGA TAG. The 21B-1 allele carries a 1-bp deletion in the middle of *BRCA2A* missing the last cytosine of the codon TCC for Ser_{453} (Fig. 2*C*). The 13G-1 allele has the same mutation as 13F-1. The mutations in both the 13F-1 and 21B-1 alleles created a premature stop codon. We examined the effects of these mutations on transcript levels by RT-PCR (Fig. S1). Complementation of these alleles was achieved with a 9.3-kb genomic fragment containing the 2-kb 5′ region, the 6.3-kb coding region, and the 1-kb 3′ region of *BRCA2A* (Fig. 2*D*).

Impact of *BRCA2* Genes on Plant Development, HR, and Defense. *Arabidopsis* is the only species known to have a second copy of the *BRCA2* gene, *BRCA2B*. The *Arabidopsis* BRCA2 proteins (BRCA2A, 1151 aa; BRCA2B, 1155 aa), which share 94%

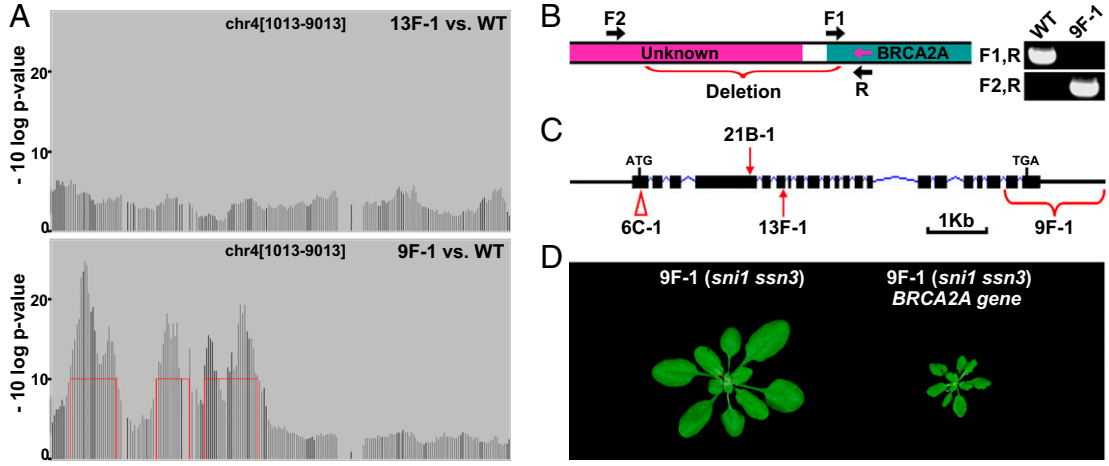

Fig. 2. Tiling array–based cloning identified the *SSN3* gene as *BRCA2A*. (*A*) The interval analysis of tiling array hybridization data. The probes are arranged in the order of their chromosomal position (top to bottom). Chr4 (1013–9013) represents the 1013- to 9013-bp region on chromosome 4. The black bars represent $-10 \times \log$ *p*-values calculated by comparing hybridization signals between mutant (allele 13F-1 or 9F-1) and WT (Col-0) to each probe. The red lines highlight the intervals with at least two consecutive probes with *P* values over the set threshold ($P = 0.1$). (*B*) Confirmation of DNA deletion in the 9F-1 allele. The schematic (*Left*) shows the 9F-1 deletion including the *BRCA2A* gene (teal bar with the pink arrow showing the gene orientation) and the unknown region (pink; not available in the *Arabidopsis* genome database) and indicates the positions of the primers used for PCR (*Right*). A PCR product was detected in WT, but not in 9F-1, using primers F1 (9F-1-F1) and R (9F-1-R) (Table S1). The size of the deletion (>10 kb) was determined using primers F2 (9F-1-F2) (the sequence was based on the results of genomic-walk) and R, from which a PCR product was generated in 9F-1, but not in WT. (*C*) Mutation positions of the four *ssn3* alleles (6C-1, 9F-1, 13F-1, and 21B-1) in the *BRCA2A* gene. ATG and TGA indicate the start and stop codons, respectively. The exons are shown as black boxes, and introns are represented by blue lines. (Scale bar: 1 kb.) In contrast to *B*, here the orientation is inverted so the *BRCA2A* gene reads from left to right. (*D*) Complementation of the *ssn3* mutant phenotype was achieved when a 9.3-kb genomic DNA fragment (*BRCA2* gene) containing the 2-kb 5′ region (upstream of the start code), the 6.3-kb BRCA2A coding region, and the 1-kb of the 3′ region (downstream of the stop code) was transformed into 9F-1 (the *sni1 ssn3* double mutant). The *sni1* morphology was observed in the transformants (9F-1 *BRCA2* gene) after 4 wk of growth in soil.

amino acid sequence identity, are only one-third the size of the human BRCA2 protein (3418 aa) (17). They are predicted to have the functional domains of the human counterpart, that is, the BRC repeats (four repeats) (18), the DNA binding domain (19), and the cyclin-binding site (20). BRCA2A and BRCA2B differ in their second BRC repeat in the core consensus amino acids; the TASGK sequence in BRCA2A is replaced by TALGK in BRCA2B (Fig. S2). With regard to gene expression, both gene transcripts are detected in floral tissues, as reported previously (17). Besides floral tissues, we found *BRCA2A* and *BRCA2B* expression in shoot and root apexes using the *BRCA2A::BRCA2A-GUS* and *BRCA2B::BRCA2B-GUS* reporters (Fig. S3*A*). We also detected a moderate level of *BRCA2A* in leaves. We found no change in *BRCA2A* and *BRCA2B* expression in the *sni1* mutant or upon SA treatment (Fig. S3*B*). When crossed into the *sni1* background, the *brca2b* mutation had little effect on the plant morphology. The effect of *brca2b* was noticeable only in the 10-d-old *sni1 brca2a brca2b* triple-mutant seedlings, in which both *brca2a* and *brca2b* are required to completely restore the WT morphology (Fig. 3*A*). Contributions of *BRCA2A* and *BRCA2B* to plant fertility are clearly redundant; the single mutants are fertile, whereas the *brca2a brca2b* double mutant is sterile (Fig. 3*B*). Vegetative growth of the double mutant is unaffected.

DNA damage, such as DNA crosslinking and double-strand breaks, can be repaired through an HR repair pathway. Because BRCA2 plays an important role in HR, we quantified the frequency of somatic recombination in the *brca2* mutants using a GUS reporter as described previously (21) and tested the mutants' responses to genotoxic chemicals. We found significant decreases in the frequency of somatic recombination in *brca2a,* but not in *brca2b* (Fig. 3*C*). Similarly, all of the *brca2a* mutant alleles were hypersensitive to mitomycin C (cross-linking DNA) and bleomycin (causing DNA double-strand breaks), whereas the *brca2b* mutants demonstrated WT sensitivity to these chemicals (Fig. 3*D* and Fig. S4).

We tested the response of *brca2* mutants to pathogen infection using the bacterial pathogen *Pseudomonas syringae* pv. *maculicola* ES4326 (*Psm* ES4326; OD_{600} = 0.0001). The *brca2a* single mutant and *brca2a brca2b* double mutant were more susceptible to infection than WT, whereas the *brca2b* single mutant was similar to WT (Fig. 3*E*), indicating that BRCA2A, but not BRCA2B, plays a role in plant defense. Consistent with the fact that *brca2a* is a suppressor of *sni1*, in the *npr1 sni1 brca2a* triple mutant, *Psm* ES4326 grew to a level similar to that seen in *npr1*; *brca2a* had only a slight effect on SA-induced resistance in the presence of NPR1, however (Fig. S5). Taken together, these findings indicate that *Arabidopsis BRCA2* genes play a broad role in plant development, defense, and HR, with *BRCA2A* as the predominant player.

RAD51, the Partner of BRCA2, Is Also an SSN. In mammals, BRCA2 is known to form a complex with RAD51 during HR through the BRC repeats (18) as well as the C-terminal region 2 (TR2) domain (20, 22). BRCA2 delivers RAD51 to double-stranded DNA breaks and guides it to selectively bind single-stranded DNA (15, 23). BRCA2 also is responsible for the removal of RAD51 once the repair is completed. The binding and release of RAD51 from BRCA2 is regulated by phosphorylation of the TR2 domain, which is coupled with progression of the cell cycle (14, 24).

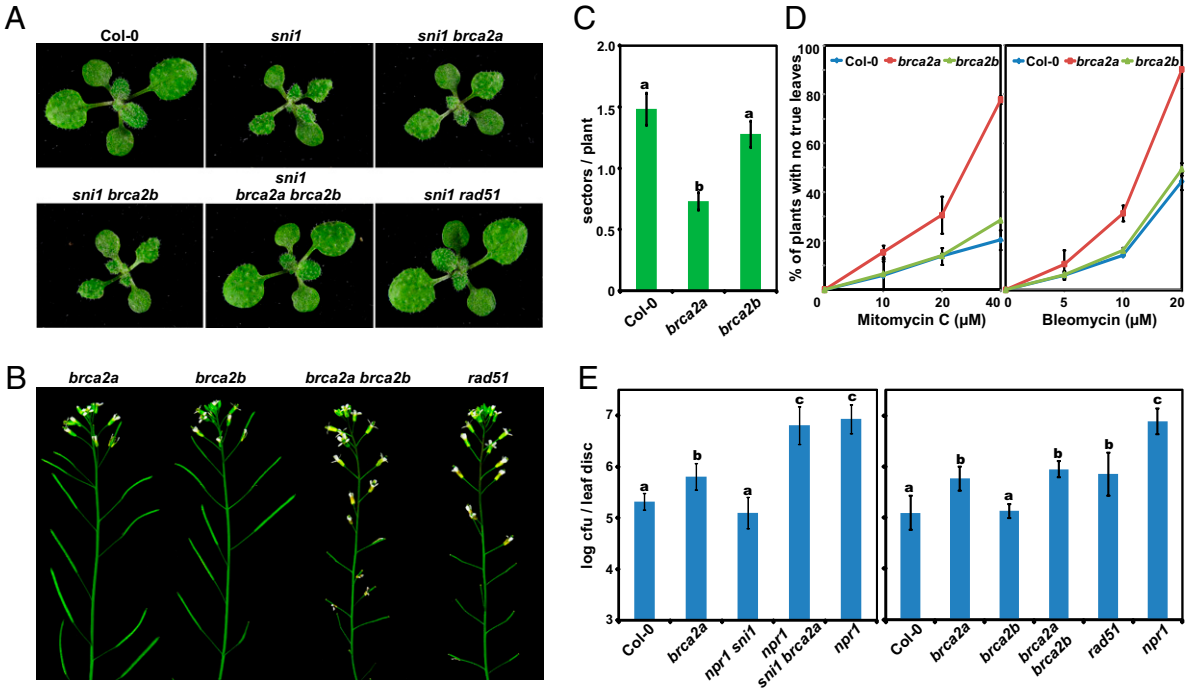

Fig. 3. BRCA2 and its partner RAD51 play a critical role in plant development, HR, DNA damage repair, and pathogen resistance. (*A*) In 10-d-old seedlings, the morphological phenotype of the *sni1* mutant was differentially suppressed by *brca2a*, *brca2b,* and *rad51*. (*B*) The impact of *brca2* and *rad51* mutations on plant fertility is shown by the presence (in *brca2a* and *brca2b*) or absence (in *brca2a brca2b* and *rad51*) of seed siliques on the inflorescence. (*C*) The frequency of somatic recombination in Col-0, *brca2a*, and *brca2b* was quantified by a *GUS* reporter (1445) (8, 21). The Student *t* test with the Bonferroni correction for multiple comparisons was performed. The letter (a or b) above the bar indicates statistically significant difference between genotypes ($P < 0.01$). (*D*) The genotoxic substances mitomycin C and bleomycin were used to treat WT (Col-0), *brca2a*, and *brca2b* plants. DNA damage is indicated by the percentage of plants with no true leaves (*y* axis). Error bars represent SEs. Experiments were conducted in triplicate (~50 plants/experiment), with similar results. (*E*) *Pseudomonas syringae* pv. *maculicola* ES4326 (OD_{600} = 0.0001) was used to infiltrate 5-wk-old plants. Bacterial growth was measured 3 d after inoculation. The error bars represent 95% confidence intervals of log-transformed data (n = 8). The Student *t* test was performed with the Bonferroni correction for multiple comparisons. The letter (a, b, or c) above the bar indicates statistically significant difference between genotypes ($P < 0.01$). Experiments were conducted in triplicate, with similar results. cfu, colony-forming units.

The BRC repeats of *Arabidopsis* BRCA2 proteins have been shown to interact with RAD51 in yeast (17), prompting us to test whether mutations in the *RAD51* gene also might be able to suppress the *sni1* phenotype. We crossed the *sni1* mutant with *RAD51*/*rad51* heterozygous plants (the *rad51*/*rad51* homozygous mutant is sterile) and found that even though the *sni1 rad51* double homozygous mutant is sterile, the vegetative developmental phenotype of the *sni1* mutant was fully restored to WT, even at the young stage (10-d-old). The effect of *rad51* on *sni1* resembled that of the *brca2a brca2b* double mutant (Fig. 3*A*). Infection tests showed that the *rad51* single mutant, similar to *brca2a*, was more susceptible to *Psm* ES4326 (Fig. 3*E*), but it had little, if any, effect on SA-induced resistance ($P = 0.07$) due to the presence of the functional NPR1 (Fig. S5). Based on these findings, we conclude that the *rad51* mutant is a suppressor of the *sni1* mutant and that the *RAD51* gene is involved in plant defense. The *rad51* mutant was not identified in our genetic screen for *ssn* mutants, probably because of its sterility.

RAD51 Protein Is Elevated in *sni1*, Induced by SA, and Interacts with BRCA2A *In Planta*. To examine how *RAD51* is regulated in the plant defense response, we also investigated the effect of the *sni1* mutation and SA treatment on the expression levels of *RAD51* as we did for *BRCA2*. In WT plants, expression of the *GUS* reporter driven by the *RAD51* promoter (*RAD51::RAD51-GUS*) is confined predominantly to the shoot and root apexes (Fig. 4*A*), similar to the *BRCA2::BRCA2-GUS* reporter genes (Fig. S3*A*). However, *RAD51* transcript levels were up-regulated both in the *sni1* mutant and by the SA treatment (Fig. 4 *A* and *B*). Because of the high homology between human and *Arabidopsis* RAD51 proteins (~70% amino acid identity) (Fig. S6), we were able to use the antibody against human RAD51 (ab48981; Abcam) to detect the *Arabidopsis* RAD51. As shown in Fig. 4*C*, in the *sni1* mutant, the RAD51 protein level was also increased as did its transcript level. Interestingly, two RAD51 protein bands in WT (Col-0) and in *sni1* were absent in *sni1 rad51*. We hypothesize that one of these bands might be modified RAD51. In the Western blots shown in Fig. 4 *C* and *D*, the lower RAD51 protein band was increased in intensity in response to SA treatment and in the *sni1* mutant background, suggesting that in the absence of an induction signal, SNI1 might play a role in repressing *RAD51* expression as well as in inhibiting protein activity by post-transcriptional modification.

In mammals, BRCA2–RAD51 complex formation is regulated through phosphorylation of the TR2 region of the BRCA2 protein (20, 24). Co-immunoprecipitation (co-IP) analysis using transgenic plants expressing *35S::BRCA2Ag-TAP* showed that BRCA2A and RAD51, like their mammalian counterparts, formed a complex *in planta* (Fig. 4*E*). How this complex is regulated in plants remains unknown, however, given that SA treatment did not affect formation of the complex.

Global Impact of *BRCA2A* on Plant Defense Gene Transcription. To investigate how the BRCA2–RAD51 complex is engaged in plant defense gene transcription during immune response, we performed the microarray analysis in the *npr1 sni1* double mutant and the *npr1 sni1 brca2a* triple mutant with and without SA induction. As shown in Fig. 5*A*, among the 989 genes with significant interactions ($P < 0.01$) between genotypes (*npr1 sni1* and *npr1 sni1 brca2a*) and treatments (water and SA), 57.45% of the changes (induction or repression) were attributed to BRCA2A. Of the 428 SA-induced genes in the *npr1 sni1* background, 61.9% were dependent on BRCA2A ($P < 0.01$; Fig. 5*B*). Gene Ontology (GO) analysis showed that defense was the most significantly enriched function in these genes (35.8%; $P < 0.01$). Remarkably, some of the key regulators and markers of the SAR signaling pathway, including *PR1*, *WRKY18*, *EDS1*, *NIMIN1*, *NDR1*, *PR5*, and *PAD4*, were among the top 20 most BRCA2A-dependent defense genes (Table S2). Our microarray data suggest that downstream or independent of NPR1, BRCA2A specifically regulates plant defense genes in response to SA induction. This conclusion is supported by the results of qPCR analyses of some defense-related genes (Fig. S7).

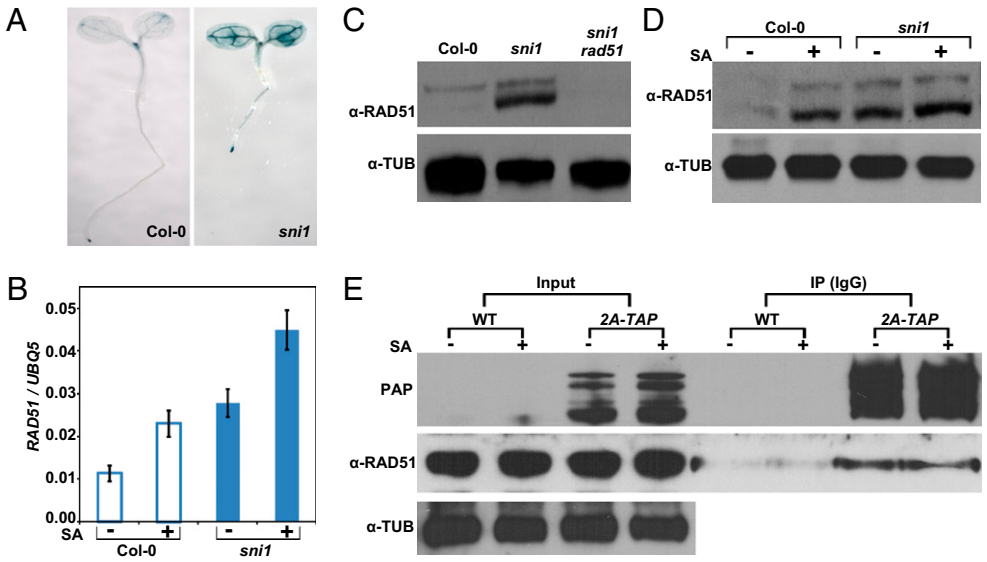

Fig. 4. *RAD51* expression and in vivo interaction between BRCA2A and RAD51. (*A*) 10-d-old WT (Col-0) and *sni1* seedlings carrying the *RAD51::RAD51-GUS* transgene were stained with X-Gluc to visualize the expression pattern of *RAD51*. (*B*) qPCR was performed to measure the endogenous *RAD51* transcription levels in WT (Col-0) and *sni1* 16 h after treatment with water (–) or 0.5 mM SA (+). The *UBQ5* transcript was used as an internal loading control. Error bars represent SEs. Experiments were conducted in triplicate, with similar results. (*C* and *D*) Total protein was extracted from 4-wk-old plants and separated using 12% SDS/PAGE gel. The resulting Western blot was probed using antibody against human RAD51 to detect the *Arabidopsis* RAD51 protein. *Arabidopsis* α-tubulin (α-TUB) was used as an internal loading control. The *sni1 rad51* double mutant was included as a negative control for the RAD51 protein (*C*). Total protein was extracted from WT (Col-0) and *sni1* plants 16 h after treatment with water or 0.5 mM SA (*D*). (*E*) RAD51 and BRCA2 form a complex *in planta*. The total protein extracts of WT and transgenic plants carrying *35S::BRCA2Ag-TAP* (2A-TAP) treated with water (–) and SA (+) served as inputs. In 2A-TAP, BRCA2A was fused in the C terminus to the TAP-tag encoded by the pYL436 vector (Arabidopsis Biological Resource Center). The proteins immumoprecipitated by IgG were blotted by antibodies against PAP, α-RAD51, and α-tubulin.

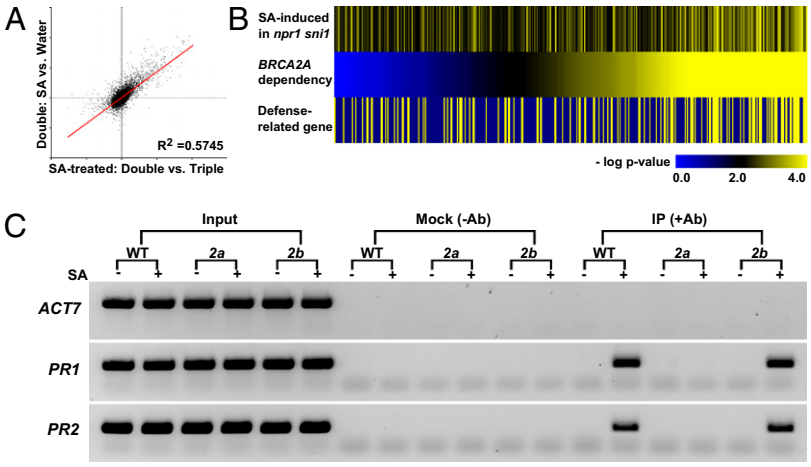

Fig. 5. Transcriptional regulation of defense genes by the BRCA2A–RAD51 complex during plant immune response. (*A*) Scatterplot and linear regression analysis showing the contribution of BRCA2A to SA-mediated gene expression. Subtracted values between the specified samples were plotted. Double refers to *npr1 sni1*; triple, to *npr1 sni1 brca2a*. (*B*) Downstream of NPR1, BRCA2A is a major regulator of the SA-induced defense gene expression. Among the 428 SA-induced genes in *npr1 sni1*, 265 were BRCA2A-dependent (yellow) and 163 were BRCA2A-independent (blue). The defense-related genes are represented by yellow bars; the others, by blue bars. The scale for the heatmaps (blue to yellow) representing −log *P* value is shown at the bottom. (*C*) SA-induced RAD51 association to *PR* gene promoters is dependent on BRCA2A. Leaf tissue was collected from 4-wk-old plants treated with water (−) or 0.5 mM of SA (+) for 16 h, fixed using 1% formaldehyde, and fractionated for nuclei to extract chromosomal complexes. The resulting samples were then subjected to sonication, followed by IP using antibody against the human RAD51 (+Ab). The chromatin precleared by the protein A agarose beads and the IP products without antibody served as input and mock (−Ab), respectively. The immunoprecipitated DNA fragments were subjected to PCR using primer pairs for the promoter regions of *ACT7* (ACT7-ChIP-3F and ACT7-ChIP-3R), *PR1* (PR1-ChIP-3F and PR1-ChIP-3R), and *PR2* (PR2-ChIP-3F and PR2-ChIP-3R) (Table S1). PCR was performed for 35 cycles. The PCR products were examined using ethidium bromide staining after agarose gel electrophoresis. *2a*, *brca2a*; *2b*, *brca2b*.

SA-Dependent Binding of the BRCA2–RAD51 Complex to *PR* Gene Promoters. We performed ChIP experiments to investigate whether BRCA2 and RAD51 are directly involved in transcription regulation of plant defense genes. We found that in WT plants, RAD51 is present at the promoters of *PR1* (−737 to −343 upstream of the start codon) and *PR2* (−549 to −254 upstream of the start codon) genes in an SA-dependent manner (Fig. 5*C*). This binding was specific to the *PR* gene promoters, as indicated by the detection of less binding or no binding in the flanking regions (Fig. S8 *A* and *B*). Little binding also was seen to the *ACT7* gene promoter (−909 to −385 upstream of the start codon), which is expressed constitutively (Fig. 5*C*). More interestingly, this binding was abolished in the *brca2a* mutant, indicating that BRCA2A is required for the specific delivery of RAD51 to the *PR* gene promoters. In contrast, the *brca2b* mutation did not affect the binding of RAD51 to the *PR* gene promoters (Fig. 5*C*), even though similar amounts of the RAD51 protein were present in *brca2a* and *brca2b* (Fig. S8*C*). This result is consistent with the minor role of BRCA2B in disease resistance (Fig. 3*E*). These findings indicate that regulating transcription of *PR* genes through interaction with RAD51 is a unique function of BRCA2A.

Discussion

The present study demonstrates that SAR-related transcription and HR are not just parallel stress responses, but involve the common protein components SNI1, RAD51D (SSN1) (8), BRCA2A (SSN3), and RAD51. The two copies of the *Arabidopsis BRCA2* genes *BRCA2A* and *BRCA2B* play overlapping as well as distinct roles in plant development, HR, and defense. The finding of *brca2a* in a genetic screen for rescuing the phenotype of *sni1* indicates the unique role of BRCA2A in plant defense. This is further validated by the enhanced susceptibility to *Psm* ES4326 infection observed in the *brca2a* mutant. In the absence of NPR1, BRCA2A function becomes essential for *PR* gene induction and disease resistance. The *npr1 sni1 brca2a* triple mutant is deficient in numerous defense-related gene transcriptions induced by SA (Fig. 5 *A* and *B*, Fig. S7, and Table S2), and thus is as susceptible to *Psm* ES4326 infection as the *npr1* single mutant (Fig. 3*E*). The difference in functionality between BRCA2A and BRCA2B is likely related to their sequence variations in the BRC repeats (Fig. S2), although possible differences in their expression patterns cannot be ruled out.

Studies in mammals have shown that BRCA2 functions through partnership with RAD51 in HR (14). In *Arabidopsis*, *rad51* rescued the phenotype of *sni1* when crossed into the *sni1* mutant background (Fig. 3*A*). As the *brca2a* mutant, mutation of the *RAD51* gene also led to enhanced susceptibility to *Psm* ES4326 (Fig. 3*E*). The direct involvement of RAD51 in plant defense is further demonstrated by the increased RAD51 protein levels detected in the *sni1* mutant, as well as after SA treatment (Fig. 4 *A*–*D*). Although whether the 35S-driven TAP-tagged BRCA2A can complement the *brca2a* mutation remains to be tested, the combined evidence based on the co-IP (Fig. 4*E*) and ChIP (Fig. 5*C*) experiments suggests the existence of an *in planta* BRCA2A–RAD51 complex.

In HR, the DNA binding of RAD51 is regulated by BRCA2 (14, 15). Our data show that the specific delivery of RAD51 to the *PR* gene promoters also requires the function of BRCA2A (Fig. 5*C*). In response to SA induction, SNI1 repression is alleviated, which might allow the BRCA2A–RAD51 complex to bind to the promoters. In mammals, phosphorylation of BRCA2 at the TR2 domain by cyclin-dependent kinase regulates this process (24). Whether phosphorylation of BRCA2A also plays a regulatory role in plants remains to be determined.

The role of BRCA2 in transcription regulation, mainly through the use of a synthetic reporter gene, has been proposed in mammals. The transcription activity of human *BRCA2* seems to depend on exon 3, which encodes a region that interacts with EMSY, an unknown protein with an 80-aa conserved domain in its amino terminus designated the ENT (EMSY N-terminal) domain and a proposed function in chromatin remodeling (25). In the presence of EMSY, the transactivation activity of the BRCA2–GAL4 fusion is reduced. This BRCA2–EMSY interaction might be biologically significant, given that the *EMSY* gene resides in an amplicon found in sporadic breast and ovarian cancers. The increase in *EMSY* level may phenocopy a deficiency in *BRCA2*. *Arabidopsis* contains nine genes encoding ENT domain–containing proteins. Besides RAD51 and EMSY, the human BRCA2 (the region encoded by exon 10) also has been found to interact with P/CAF, which has the activity of histone acetyltransferase (26). The biological process for which this BRCA2 function is required remains unknown, however.

Our molecular genetic data clearly show that in *Arabidopsis*, the BRCA2A–RAD51 complex not only plays a role in HR, but also is specifically involved in the transcriptional regulation of defense-related genes during SAR. The former function occurs randomly in the chromosomes, whereas the latter is signal- and promoter-specific. The specificity of RAD51's association with the *PR* gene promoters might be dependent on a transcription factor at the *PR* gene promoters but likely is independent of the

transcription cofactor NPR1, given the fact that in the *npr1 sni1* double mutant, the *PR* genes can still be induced by SA, albeit at lower levels (Fig. 1*B*, Fig. S7, and Table S2).

The involvement of the BRCA2A–RAD51 complex in defense-related gene transcription may have intriguing biological significance. We hypothesize that along with its function in remodeling chromatin to facilitate transcription, the BRCA2A–RAD51 complex also might play a role in safeguarding against transcription-associated DNA instability (12). SAR induction can alter the expression of as many as 10% of *Arabidopsis* genes (2). This transcription reprogramming and associated chromatin remodeling make the genome more vulnerable to genotoxic agents, such as reactive oxygen species, which are released during plant immune responses. A recent study in a human cell line showed that gene-specific double-stranded DNA breaks can be induced by nuclear receptors and several other transcription factors (27). Our discovery that the *Arabidopsis* BRCA2–RAD51 complex plays a specific role in transcription regulation during a plant immune response may spark future searches for the specific physiological process regulated by this complex in humans and address the question of why a BRCA2 deficiency predisposes to cancer specifically in the breast and ovary.

Materials and Methods

Full details are provided in *SI Materials and Methods*.

Plant Material and Transformation. The *brca2b* mutant (SALK_037617) was obtained from the Arabidopsis Biological Resource Center. The *rad51* mutant is GABI_134A01 (28). *Arabidopsis* transformation was performed as described previously (29).

Mutant Screen. The genetic screen was carried out as described previously (8).

Tiling Array-Based Cloning. DNA was labeled using the BioPrime DNA Labeling System (Invitrogen) and hybridized with the GeneChip *Arabidopsis* Tiling 1.0R Array (Affymetrix). The chip data were analyzed using GeneChip Tiling Analysis Software (Affymetrix).

GUS Reporter Analysis The *BGL2::GUS* reporter was used to detect SA response (3).

HR Measurement. A reporter containing overlapping segments of the *GUS* gene in inverted orientation (line 1445) was used for HR measurement, as described previously (8, 21).

***Pseudomonas* Infection.** *Pseudomonas* infection was carried out as described previously (8).

Treatment of Genotoxic Substances. Mitomycin C and bleomycin treatments were performed as described previously (8).

qPCR and RT-PCR. qPCR was performed using the QuantiTect SYBR Green PCR Kit (Qiagen) in a LightCycler Real-Time PCR System (Roche Applied Science). The primers used for qPCR and RT-PCR are listed in Table S1.

Protein Analysis. Western blot analysis was performed as described previously (4). Co-IP was performed using IgG Sepharose 6 Fast Flow (GE Healthcare).

Microarray Analysis. RNA was labeled using the MessageAmp Premier RNA Amplification Kit (Ambion) and hybridized with the GeneChip *Arabidopsis* ATH1 Genome Array (Affymetrix). Microarray data values were normalized using GeneSpring GX Software (RMA algorithm; Agilent).

ChIP. ChIP was carried out as described previously (30). Chromatin was sonicated at 40% duty for 4 × 10 s with a Branson Digital Sonifier 250.

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