DNA Repair Proteins Are Directly Involved in Regulation of Gene Expression during Plant Immune Response

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SUMMARY

Systemic acquired resistance (SAR), an inducible plant-defense response to local infection, requires the signaling molecule salicylic acid (SA) and the transcriptional coactivator NPR1, with concerted activation of pathogenesis-related (PR) genes. Arabidopsis sni1 is an npr1 suppressor and derepression of defense genes in sni1 causes reduced growth and fertility and increased homologous recombination. Characterizing suppressors of sni1, we identify the DNA damage repair proteins SSN2 and RAD51D as genetic and physical interactors with SN11. During plant defense, SSN2 and possibly RAD51D replace the transcription repressor SN11 at pathogenesis-related gene promoters. In the presence of SN11, NPR1 is also required for SSN2 binding. Thus, coordinated action of SN11, SSN2-RAD51D, and NPR1 ensures the tight control of plant immune gene expression. Given that the SSN2-RAD51D complex is conserved in eukaryotes, their dual function in homologous recombination and transcription regulation of plant-defense genes suggests a general link between these two stress responses.

INTRODUCTION

Plants are constantly exposed to a wide range of microbial pathogens in nature. They have evolved effective defense mechanisms to cope with infection (Jones and Dangl, 2006). Systemic acquired resistance (SAR) is an inducible defense response that occurs after local infection and provides long-lasting, broad-spectrum resistance to secondary infection (Durrant and Dong, 2004; Ryals et al., 1996). The onset of SAR requires local and systemic increases of the signal molecule salicylic acid (SA), accompanied by concerted activation of pathogenesis-related (PR) genes. These genes encode small secreted or small vacuole-targeted proteins with antimicrobial activities. In particular, PR1 and β-1,3-glucanase (BGL2, also called PR2) are well-used molecular markers of SAR (Uknes et al., 1992).

Constitutive activation of defense is usually detrimental to plant growth and development and therefore both temporal and spatial expression of PR genes are strictly regulated at multiple levels. Mutants with elevated expression of defense genes often have decreased plant fitness associated with reduced stature, loss of apical dominance, and decreased fertility (Bowling et al., 1997; Dietrich et al., 1994; Greenberg et al., 1994). The inducible nature of SAR allows plants to activate defense only when the benefit of resistance outweighs the cost on growth and development. Previously in a genetic screen for npr1 suppressors sni1 was identified, which restored the inducibility of PR gene expression in the npr1 background (Li et al., 1999). SN11 encodes a leucine-rich nuclear protein and does not share any obvious homology with known proteins in the database. Structural similarity searches revealed that SN11 may function as a scaffold for interaction with various signaling proteins (Moshier et al., 2008). However, identification of potential interactors with SN11 by yeast two-hybrid analysis was hampered by its transcriptional repressor activity (Moshier et al., 2006). The underlying molecular mechanism of how SN11 regulates defense response remains elusive.

In this study, we identified a suppressor of sni1, ssn2, which encodes a protein containing a SWIM (SWI2/SNF2 and M uDR) domain found in a variety of prokaryotic and eukaryotic proteins. We present compelling evidence that SN11 forms complexes with both SSN2 and RAD51D, which are previously known partners in homologous DNA recombination (Durrant et al., 2007; Martin et al., 2006). SN11 binds to the PR1 gene promoter to suppress defense response in the absence of pathogen challenge and is removed in response to induction. Our data suggest that the positive regulators SSN2 and RAD51D are recruited to the PR1 gene promoter through interaction with the TGA7 transcription factor and by the function of the transcription cofactor NPR1. Our findings reveal a previously uncharacterized function of the SSN2-RAD51D complex in direct transcriptional regulation and demonstrate that multicellular organisms employ these proteins to modulate their transcriptional responses to external stimuli.

RESULTS

SSN2, Suppressor of sni1 2, Is a Positive Regulator of Plant Defense

The identification of rad51d (ssn1) as a mutant compromised in plant-defense gene expression showed that a DNA repair protein can play a role in transcription (Durrant et al., 2007). To investigate whether this is an isolated association or there is a deeper connection between these two stress responses, we
studied another mutant, ssn2, isolated in the same genetic screen for suppressors of snl1. The snl1 single mutant exhibits pleiotropic phenotypes, such as early flowering, reduced fertility, short roots, and narrow leaves. In the snl1 mutant, several PR defense marker genes show elevated background expression (Li et al., 1999). The suppressor screen was designed to search for mutants that resembled wild-type in morphology and had abolished the background expression of PR genes in snl1. As shown in Figure 1A, ssn2 restored wild-type morphology to both snl1 and snl1pnr1. The elevated background expression of the SA-responsive BGL2:GUS reporter in snl1 and snl1pnr1 was also suppressed in ssn1ssn2 and ssn1pnr1ssn2. Moreover, in snl1pnr1ssn2, the reporter gene lost its responsiveness to exogenous application of a SA functional analog INA (2,6-dichloronicotinic acid) (Figure 1A). A time course expression analysis performed on the PR1 gene also showed a significant delay in gene induction in the ssn2 single mutant (Figure 1B).

To determine whether the effect of ssn2 is restricted to BGL2:GUS and PR1 or general to the transcription reprogramming during plant defense, we performed microarrays on snl1pnr1 and snl1pnr1ssn2 with and without SA treatment. As shown in Figure 1C, among the 270 genes significantly induced in snl1pnr1 (p value < 0.05), 147 (54.4%) were dependent on Ssn2 (Document S2, available online). Gene ontology analysis found 31 defense-related genes in the Ssn2-dependent group (21.1%) and 14 in the Ssn2-independent group (11.4%). We also performed qPCR on nine well-known defense genes detected in the microarray and found that SA induction of genes such as PR1, PR2, PR5, EDS1, and PAD4 was diminished by the ssn2 mutation (Figure S1A).

The ssn2 mutation was mapped to the At4g33925 locus (Figure S1B). Through sequencing analysis, a 3.4 kb gypsy-like retrotransposon insertion was identified in the first exon of At4g33925 in ssn2-1 (Figures S1C and S1D). A ~40 kb deletion and a large DNA rearrangement were found in the chromosomal region of At4g33925 for ssn2-2 and ssn2-3, respectively. The ssn2-1 allele was selected for further experiments and is hereafter referred to as ssn2. To confirm that At4g33925 is Ssn2, we transformed the genomic DNA containing the entire At4g33925 gene into snl1ssn2, and the resulting homozygous transgenic lines restored the snl1 morphology and the background BGL2:GUS expression (Figure 1A).
SSN2 encodes a small protein with one recognizable domain known as the SWIM domain, which has been shown to be involved in both DNA binding and protein-protein interaction in different proteins (Makarova et al., 2002; Martín et al., 2006). Homologs of SSN2 have been found in all eukaryotes (Martín et al., 2006). Confocal fluorescence microscopic examination of transgenic plants expressing a functional 3SS:SSN2-GFP transgene showed that SSN2 was predominantly localized in the nucleus (Figure S1E), similar to SN1 (Mosher et al., 2006). By using a fusion of the SSN2 promoter to the GUS reporter, SSN2 gene expression was detected in leaves, predominantly in shoot apexes. Moderate expression of SSN2 was also observed in roots (Figure S1F).

To evaluate the biological function of SSN2 in plant-defense response, we challenged the ssn2 mutant with *Pseudomonas syringae* pv. *maculicola* ES4326 (Psm ES4326). As shown in Figure 1D, there was an increase in growth of Psm ES4326 in the ssn2 single mutant. This defect in resistance was more pronounced in the npr1:ssn2 double (Figure S1G) and ssn1npr1:ssn2 triple (Figure 1D) mutants. To monitor the dynamic change of SSN2 protein levels in response to SA, we generated stable transgenic lines containing the SSN2-TAP (tandem affinity purification) fusion construct driven by the native SSN2 promoter. To confirm the functionality of SSN2:SSN2-TAP, we crossed a transgenic line with ssn1:ssn2 and the homozygous ssn1:ssn2 progeny containing SSN2:SSN2-TAP regained the ssn1 phenotype, indicating that the SSN2-TAP fusion protein is biologically active (Figure S1H). The SSN2:SSN2-TAP lines were treated with 0.5 mM SA and the SSN2-TAP fusion protein was analyzed at different time points. As shown in Figure 1E, levels of SSN2-TAP protein were upregulated by SA with the highest expression at 8 hr after treatment. This SA-inducible nature of the SSN2 protein, together with the disease phenotype of the ssn2 mutant, suggests that SSN2 plays a positive role in defense responses.

**SSN2 Physically Interacts with SN1**

In view of the genetic interactions between SSN2, RAD51D (SSN1) (Durrant et al., 2007), and SN1, we explored the possibility of physical interactions between these proteins by using yeast two-hybrid analysis. Because the SSN1 protein exhibited transcriptional repressor activity in yeast (Mosher et al., 2006), both full-length and various truncations of the SN1 gene that were made according to previous functional analysis of SN1 (Mosher et al., 2006) were cloned into the GAL4 yeast two-hybrid (Y2H) vectors to test interactions. As shown in Figure 2A, strong interaction was revealed between the full-length SN1 and SSN2. Three SN1 truncations containing the central region (aa114–233) also interacted with SSN2, suggesting that this part of SN1 is required for mediating the interaction with SSN2 (Figure 2A). SSN2 contains the SWIM domain, consisting of a CxChC,CxH motif located downstream of two predicted β strands and followed by an α helix (Makarova et al., 2002). To examine the functional significance of the SWIM domain, we substituted the first conserved cysteine of this motif with a serine residue (C82S). As shown in Figure 2A, the interaction with SN1 was reduced by the ssn2-C82S mutation. Immunoblot analysis confirmed that similar levels of ssn2-C82S and wild-type SSN2 proteins were produced in these yeast strains (Figure S2A).

**Deficiency in SSN2 Leads to Hypersensitivity to DNA-Damaging Agents**

To determine whether the *Arabidopsis* SSN2 has any function in DNA damage repair, we examined the ssn2 mutant plants for somatic recombination by using an in planta recombination assay with a reporter construct containing two overlapping fragments of the *GUS* gene (Lucht et al., 2002). Recombination events between the two overlapping sequences produce a functional GUS gene resulting in blue clusters of cells, which can be visually detected after histochemical staining. The ssn2 mutant was crossed with the recombination reporter line and the frequency of homologous recombination was measured in the F3 progeny homozygous for the GUS reporter in the ssn2 background. As shown in Figure 3A, the frequency of homologous recombination in the ssn2 mutant was reduced by half compared to that of the wild-type plant. Furthermore, the ssn2 mutant also showed a reduced rate of recombination under INA-induced condition, indicating that SSN2, like Sws1 in yeast, plays a role in homologous DNA recombination.

The inability to properly repair DNA damage often leads to increased sensitivity to a variety of DNA-damaging agents (Bray and West, 2005). Mitomycin C (MMC) is an alkylating agent which induces DNA interstrand crosslinking. Plants sensitive to DNA damage agents fail to develop true leaves. Nearly 20% of the ssn2 mutant plants failed to develop true leaves on MS.
aggar medium containing 20 μM MMC, whereas wild-type plants produced normal true leaves at this concentration (Figure 3B). We also tested the sensitivity of the ssn2 mutant to bleomycin, a γ-ray-mimicking agent which can cause double-strand breaks. As expected, the ssn2 mutant is also hypersensitive to bleomycin in comparison with the wild-type plant (Figure 3B) suggesting an important role of Ssn2 in DNA damage repair that is similar to RAD51D (Durrant et al., 2007). **SN1 and Ssn2 Have Inverse Binding Activities to the PR1 Gene Promoter** The DNA damage repair and defense phenotypes detected in sn2 and rad51d (Durrant et al., 2007) lead us to ask how the Ssn2-Rad51D repair complex affects plant immunity. The compromised basal expression of the BGL2:GUS reporter in sn1ussn2 (Figure 1A) and sn1rad51d (Durrant et al., 2007), the delayed expression of the PR1 gene (Figure 1B) in the sn2 single mutant, and the significant blockage in SA induction of defense genes observed in sn11np1ssn2 by microarray (Figure 1C) suggest that the Ssn2-Rad51D complex may affect defense at the transcriptional level. To determine whether SN1 and Ssn2-Rad51D are directly involved in repressing and activating PR gene expression, respectively, we performed a series of chromatin immunoprecipitation (ChIP) experiments by using a set of probes covering both the promoter and the coding regions of PR1 (Figure 4A). Probes were chosen according to previous promoter studies. Specifically, probe 2 contains a region that when present in the promoter reduced both the basal expression and the inducibility of the gene (Lebel et al., 1998), suggesting that it contains a negative cis-acting element. Because this region contains a W-box, it is possible that one of the WRKY transcription factors that are known to negatively regulate PR gene expression (Kim et al., 2008; Wang et al., 2006; Xu et al., 2006) functions through this promoter element. Probe 3 contains two as-1 elements (L5S and L5T) and one W-box (LS4), which have been shown through linker-scanning mutagenesis to be positive and negative elements, respectively (Lebel et al., 1998). Ampere evidence showed that TGA transcription factors regulate PR1 expression through these as-1 elements (Johnson et al., 2001; Lam and Lam, 1995; Niggeweg et al., 2000). Probes 1 (−2116 to −1829 bp), 4 (−508 to −282 bp), and 5 (−340 to +429 bp) cover regions not known to affect transcription and therefore serve as negative controls.

To study the SN1 protein binding, we generated a transgenic line containing the SN11-TAP construct under the control of the native SN1 promoter in the sn11 background. The functionality of this fusion protein was confirmed by restoration of wild-type
morphology (Figure S3A). We first examined SNI1-TAP protein levels in response to 0.5 mM SA treatment and found that they remained constant after SA treatment suggesting that the SNI1 protein is not regulated by changes in protein levels (Figure 4B). ChIP experiments revealed significant enrichments of the probe 2 promoter region comparing the SNI1-TAP sample with the untransformed wild-type plant (WT) (Figure 4C) indicating that SNI1 is associated with the PR1 promoter in a region that contains a negative cis-acting element (e.g., W-box) (Lebel et al., 1998). Moreover, comparing the water-treated SNI1-TAP sample with the SA-treated sample (Figure 4D), we found that the protein binding was reduced upon SA induction. Similar results were obtained by using a transgenic line (SNI1:SNI1-GFP) expressing the GFP-tagged SNI1 (Figures S3B and S3C). These data are consistent with SNI1’s role as a transcription repressor. Because sni1 was identified as a suppressor of npr1, it is possible that NPR1 activates PR1 by removing SNI1 from the promoter. To test this hypothesis, we carried out ChIP by using SNI1:SNI1-TAP in the sni1npr1 double mutant. We found that removal of SNI1 from the PR1 promoter is NPR1 independent (Figure 4E), indicating that a different mechanism is utilized by NPR1 to negate SNI1 repression.

The association of SNI1 with the PR1 promoter suggests that its suppressor SNS2 may also function at the promoter. To perform ChIP with SNS2, we utilized the SNS2:SNS2-TAP lines. As shown in Figure 5A, SNS2 was recruited to the PR1 promoter in response to SA induction. The binding region defined by ChIP contains probe 2 with the negative element and probe 3 with two of the TGA transcription factor binding as-1 elements, which are essential for the SA-responsiveness of this gene (Lebel et al., 1998). Because the SNS2 protein level was very low and the enrichment observed was moderate, we performed additional ChIP by using the HA-tagged SNS2 with similar results (Figure S4A).

SSN2 Promoter Binding May Require NPR1 and TGA7

Because NPR1 is not required for SNI1’s removal from the promoter, we investigated the possibility that NPR1 is required for the recruitment of SNS2. We first performed ChIP analysis on NPR1 by using the 35S:NPR1-GFP transgenic lines to determine the promoter region to which NPR1 binds. As shown in Figure 5B, we detected a significant enrichment of the probe 3 region in the SA-treated sample. The NPR1 ChIP experiment was further validated by the finding that NPR1 was also recruited to the promoter of WRKY18, which is another direct target of NPR1 upon induction (Figures S4B and S4C) (Wang et al., 2005). Because both NPR1 and SNS2 bind to the same promoter regions, we then investigated whether SNS2 promoter binding requires NPR1. ChIP assay performed in the npr1 mutant background showed that SNS2 could no longer bind to the PR1 promoter under induced condition, indicating that the recruitment of SNS2 is dependent on NPR1 (Figure 5C).

This dependency on NPR1 is unlikely to be due to a physical interaction between SNS2 and NPR1, as neither Y2H analysis nor in vivo pull-down experiments showed a positive result. Therefore, we investigated the possibility that SNS2 interacts directly with members of TGA transcription factors, as NPR1 is a known cofactor of TGAs (Després et al., 2000; Zhang et al., 1999; Zhou et al., 2000). In a Y2H analysis, NPR1 interacted with all the TGAs except TGA4 (Tada et al., 2008) and SNS2 showed interaction with only TGA7 (Figure 6A). This interaction was weakened by the snn2-c825 mutation in the SWIM domain of SNS2. In contrast, RAD51D failed to interact with TGA7 (Figure 6A). Furthermore, the tga7 mutant showed enhanced susceptibility to Psm ES4326 compared to the wild-type plant (Figure 6B), consistent with the idea that TGA7 acts as a transcription activator of PR genes. The association between SNS2 and TGA7 was demonstrated in vivo by coimmunoprecipitation when SNS2-myct and TGA7-YFP were transiently expressed in N. benthamiana (Figure 6C). TGA7-YFP could pull down SNS2-myct, whereas the negative control, GFP protein alone, failed to do so (Figure S5). To determine whether the SNS2 binding to the PR1 promoter is dependent on TGA7, we introduced the SNS2:SNS2-TAP construct into the tga7 background by crossing and then carried out ChIP assay in the homozygous progeny. As shown in Figure 6D, no significant enrichment was observed for any of the promoter regions. Therefore, the promoter specificity of SNS2 may be defined through its interaction with the TGA7 transcription factor and depend on the function of the cofactor NPR1.

DISCUSSION

Although genetic data showed that SNI1 negatively regulates defense response, its molecular mechanism has not been clearly defined (Mosher et al., 2006). In this study, we provide evidence that SNI1 suppresses PR1 gene expression through association with the PR1 gene promoter region that contains a W-box (Figure 7). Because no discernible DNA binding domain has been identified in the SNI1 protein, the binding may be indirect and perhaps mediated by a WRKY transcription factor that plays a negative role in defense signaling. WRKY18, WRKY40, and WRKY60 have been shown to function redundantly and
cooperatively to suppress the expression of some defense-related genes (Xu et al., 2006). Moreover, WRKY38 and WRKY62 were found to negatively regulate plant-defense response through interaction with histone deacetylase 19 (Kim et al., 2008). Whether and which WRKY transcription factor associates with SN11 will be the subject of further investigation.

The repressor function of SN11 cannot be explained solely by its occupancy of the promoter region to which positive regulators such as SSN2 bind because in the npr1 mutant SN11 can still be removed from the promoter upon induction while no PR1 is transcribed. Therefore, we hypothesize that SN11 binding to the promoter may bring about a chromatin structure that is unfavorable for transcription, perhaps through recruitment of a chromatin-remodeling protein such as a histone deacetylase (shown as X in Figure 7). This closed chromatin structure is reversed by NPR1, perhaps partly through the activity of SSN2.

The physical interaction between SSN2 and TGA7 both in yeast and in planta suggests that SSN2 is recruited to the PR gene promoter through TGA7, which is known to bind to the same promoter elements in electrophoretic mobility shift assay (Shearer et al., 2009). Interestingly, of the TGA transcription factors, TGA7 is the only one that interacts with SSN2 in Y2H analysis. This may explain the significant disease susceptibility phenotype observed in the tga7 single mutant. However, tga7 is not as susceptible as npr1 because of the presence of other TGA s, which are able to induce PR1 expression in the presence of a W-box but not in the as-1 element.
of NPR1. Moreover, SA-induced recruitment of SSN2 is NPR1 dependent. Therefore, either activation of TGA by NPR1 or NPR1-dependent SNI1 inactivation and SSN2 binding at the promoter region is sufficient to cause PR1 gene induction. In the absence of SNI1, the chromatin is more accessible and NPR1 is no longer required for SSN2 binding and PR1 gene transcription. This is consistent with previous promoter linker-scanning studies demonstrating that the PR1 promoter contains multiple positive and negative cis-acting elements (Lebel et al., 1998).
SSN2-RAD51D complex may activate defense gene expression by opening chromatin via the action of a protein like RAD54.

The homologous recombination pathway has been well-characterized in yeast and mammalian systems. We have uncovered a previously unknown function of these DNA repair proteins in direct regulation of gene transcription in response to infection. Our data illustrate that complex mechanisms are involved in coordinating the actions of negative regulator SN1, positive regulator SSNs, and coactivator NPR1 to achieve tight control on plant immune genes. Because the components of SSN2-RAD51D complex, as well as their interaction, are conserved in eukaryotes from fission yeast to humans and plants, our discovery of their role in transcription may have broad implications in general biology.

**EXPERIMENTAL PROCEDURES**

**Gene Expression Analysis**
Total RNA was extracted from 3-week-old soil-grown plants treated with 0.5 mM SA at different time points. First-strand cDNA was synthesized by using the Superscript™III reverse transcriptase (Invitrogen). Quantitative PCR was carried out on cDNA diluted 20 times by using QuantiTect SYBR Green PCR Kit (Qiagen) in a LightCycler (Roche) according to the manufacturer’s instructions. Gene expression analysis was performed as previously described with cDNA samples normalized by using ubiquitin5 (Kesarwani et al., 2007). The primer sequences used for qPCR are listed in Table S1.

**Microarray Analysis**
Total RNA was extracted from 3-week-old plants 16 h after treatment with water or 0.5 mM SA. The RNA labeled with MessageAmp Premire RNA Amplification Kit (Ambion) was hybridized with GeneChip Arabidopsis ATH1 Genome Array (Affymetrix) and subsequently washed and scanned at the Duke Microarray Facility. Experiments were carried out in triplicate. The microarray data were normalized by using Gene-Spring GX Software (RNA algorithm: Agilent). The two-way ANOVA with Benjamini-Hochberg multiple comparison correction was used to identify SSN2-dependent genes (significant interaction between genotypes and treatments, p < 0.01). The SA-induced genes were found through unpaired Student’s t test with Benjamini-Hochberg multiple comparison correction. The gene ontology analysis was performed by using the corresponding module in Gene-Spring.

**Bacterial Infection Assay**
Infection of Arabidopsis plants with Pseudomonas syringae pv maculicola ES4326 was performed as described previously (Durant et al., 2007).

**Yeast Two-Hybrid Analysis**
Yeast two-hybrid analysis was performed by using the Matchmaker Gold system following the manufacturer’s instructions (Clontech). Bait cDNAs were cloned into the pGBKKT7 vector and transformed into yeast strain Y187 (MATa). Prey cDNAs were cloned into the pGADT7 vector and transformed into yeast strain AH109 of the opposite mating type (MATa). Protein-protein interaction was determined by growth rate of mating yizytes on SD-Leu-Trp-His-Ado plates.

**Coimmunoprecipitation**
The cDNAs of SSN1, SSN2, RAD51D, and TGA7 were cloned into the pMOD8 (GFp), pGW2020 (10 X myc), or pET101 (YFP) vectors to generate SSN2-GFP, SN1-myc, RAD51D-myc, SN1-YFP, TGA7-YFP, and SSN2-myc constructs, which were transformed into Agrobacterium tumefaciens strain GV3101. For transient protein expression in N. benthamiana, Agrobacterium cultures were infiltrated into 4-week-old plants. Leaves were collected and processed 3 days later. For the coimmunoprecipitation experiment with SSN2 and RAD51D, the leaf tissues were crosslinked with 2 mM dithiothreitol (sucinimidyldi propionate) (DSP; Pierce) for 30 min at room temperature to stabilize protein-protein interaction. The crosslinking reaction was quenched by
addition of 1M Tris to a final concentration of 50 mM. Protein extraction was carried out by homogenizing leaf tissue in extraction buffer (50 mM Tris·HCl [pH 7.5], 150 mM NaCl, 5 mM EDTA, 0.1% Triton X-100, 10% glycerol, 40 μM MG115, and protease inhibitor cocktail at 1:100 [Sigma–Aldrich]). Homogenates were centrifuged three times at 16,000 g for 10 min at 4°C. Immunoprecipitation was performed using the anti-myct agarose (Sigma–Aldrich) or the anti-GFP antibody (Abcam) followed by incubation with Dynabeads protein G (Invitrogen). The precipitated protein eluted with the SDS loading buffer and 1% of the total protein used as input were subjected to SDS-PAGE and immunoblotted with various antibodies.

In Planta Recombination Assay
The recombination reporter line 1445 containing overlapping segments of the US gene in inverted orientation (Luch et al. 2002) was crossed into the ant1-sns2 mutant carrying the BGL2:US transgene. A homozygous line containing the recombination reporter gene in the sns2 single mutant background was isolated and recombination frequencies were measured as previously described (Durrant et al., 2007).

Mitomycin C and Bleomycin Sensitivity Assay
Plants were grown on MS media containing different concentrations of mitomycin C (0, 10, 20, or 30 μM) or bleomycin (0, 5, 10, or 20 μg/mL) (Sigma–Aldrich) for 14 days and scored for production of true leaves. Sensitivity was indicated by the percentage of plants without true leaves.

Chromatin Immunoprecipitation
ChIP assay was performed as described previously (Gendrel et al., 2005). Briefly, 1 g of 9- to 12-day-old Arabidopsis seedlings grown on MS plates treated with water or 0.5 mM SA for 8 or 16 hr was crosslinked with 1% formaldehyde under vacuum for 15 min at room temperature. The crosslinking reaction was stopped by adding glycine to a final concentration of 0.125 M. The seedlings were washed with water and then ground in liquid nitrogen. Arabidopsis nuclei were isolated and sonicated to shear DNA into 500 to 1000 bp fragments by using Branson Digital Sonifier 250 (Branson Ultrasone) followed by immunoprecipitation. For the SNS2-TAP and SN1-TAP proteins, IgG Sepharose 6 Fast Flow beads (GE Healthcare Biosciences) were used. For the GFP-tagged proteins, anti-GFP (Abcam) was first coupled to protein G Dynabeads (Invitrogen) and then used for immunoprecipitation. The purified ChIP samples were subject to qPCR analysis. To normalize the results, we quantified the amount of 18S rRNA gene sequence as the internal control. Fold enrichment was determined by the relative ratio of the normalized ChIP signals between samples. The primer sequences used for ChIP are listed in Table S1.

ACCESSION NUMBERS
The microarray data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database (accession number GSE23617).

SUPPLEMENTAL INFORMATION
Supplemental Information includes five figures, one table, Supplemental Experimental Procedures, and one data file and can be found with this article online at doi:10.1016/j.chom.2011.01.011.

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