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Activation of an EDS1-Mediated *R*-Gene Pathway in the *snc1* Mutant Leads to Constitutive, NPR1-Independent Pathogen Resistance

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The Arabidopsis NPR1 protein is an essential regulatory component of systemic acquired resistance (SAR). Mutations in the *NPR1* gene completely block the induction of SAR by signals such as salicylic acid (SA). An Arabidopsis mutant, *snc1* (suppressor of *npr1-1*, constitutive 1), was isolated in a screen for suppressors of *npr1-1*. In the *npr1-1* background, the *snc1* mutation resulted in constitutive resistance to *Pseudomonas syringae maculicola* ES4326 and *Peronospora parasitica* Noco2. High levels of SA were detected in the mutant and shown to be required for manifestation of the *snc1* phenotype. The *snc1* mutation was mapped to the *RPP5* resistance (*R*) gene cluster and the *eds1* mutation that blocks *RPP5*-mediated resistance suppressed *snc1*. These data suggest that a *RPP5*-related resistance pathway is activated constitutively in *snc1*. This pathway does not employ NPR1 but requires the signal molecule SA and the function of EDS1. Moreover, in *snc1*, constitutive resistance is conferred in the absence of cell death, which is often associated with *R*-gene mediated resistance.

Disease resistance in plants against microbial pathogens can be achieved through a number of mechanisms. Recognition of an avirulence signal from the pathogen by a cognate plant resistance (*R*) gene product often leads to a localized hypersensitive response (HR), which is defined by rapid cell death at the site of infection, leading to the restriction of pathogen colonization. During the HR, cell death is accompanied by several active physiological responses, including ion fluxes, production of reactive oxygen species, and accumulation of salicylic acid (SA) (Hammond-Kosack and Jones 1996). As a result of these responses, proliferation of the pathogen is halted at the site of infection. In recent years, many *R* genes that confer resistance against different pathogens have been cloned from a variety of plant species (Baker et al. 1997; Bent 1996; Staskawicz et al. 1995). Intriguingly, the conservation of protein domains has been found in most of the *R* gene products, indicating that the products may signal through common downstream components. Indeed, such downstream components have been identified (Century et al. 1995; Parker et al. 1996; Shirasu et al. 1999; Warren et al. 1999). The Arabidopsis NDR1 protein is essential for the resistance response mediated by several *R* gene products of the LZ-NB-LRR class, which contain a putative leucine zipper (LZ), a nucleotide binding site (NB), and leucine-rich repeats (LRR) (Aarts et al. 1998). The Arabidopsis EDS1 protein, however, is required for the resistance response specified by the TIR-NB-LRR class of *R* gene products (Aarts et al. 1998; Parker et al. 1996). Instead of the LZ domain, these *R* gene products contain a TIR domain, which is homologous to the Drosophila Toll protein and mammalian IL-1 receptors.

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The *R* gene-mediated HR often triggers a secondary resistance response known as systemic acquired resistance (SAR) (Kuc 1982; Ross 1961; Ryals et al. 1996). SAR provides the plant with long-lasting protection against a broad spectrum of pathogens. The mechanism of SAR has been studied extensively in recent years (Dong 1998; Ryals et al. 1996). An increase in the endogenous levels of SA after a HR has been shown to correlate with the onset of SAR (Malamy et al. 1990; Métraux et al. 1990; Rasmussen et al. 1991). The bacterial *nahG* gene, which encodes the SA-degrading enzyme salicylate hydroxylase, was used to establish the essential role of SA in SAR. Expression of *nahG* in transgenic plants prevents the accumulation of SA and blocks the induction of SAR (Gaffney et al. 1993). *nahG* transgenic plants also are compromised in certain *R* gene-mediated resistance (Clarke et al. 2000; Delaney et al. 1994; Nawrath and Métraux 1999). Exogenous application of SA or its analogs such as 2,6-dichloroisonicotinic acid (INA) and benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester is sufficient to induce SAR (Görlach et al. 1996; Métraux et al. 1991; White 1979).

The expression of many pathogenesis-related (*PR*) genes also is associated with SAR (Uknes et al., 1992; Van Loon and Van Kammen 1970; Ward et al. 1991; Yalpani et al. 1991). Even though the expression of these genes is up regulated during SAR, their roles in conferring resistance have yet to be established firmly. Nevertheless, *PR-1* and β-1,3-glucanase (*BGL2*, also known as *PR-2*) have been used widely as molecular markers of SAR (Bowling et al. 1994; Cao et al. 1994; Uknes et al. 1992).

Genetic analysis of Arabidopsis mutants with altered SAR has led to the identification and cloning of a positive SAR regulator, *NPR1* (also known as *NIM1*) (Cao et al. 1994; Cao et al. 1997; Delaney et al. 1995; Glazebrook et al. 1996; Ryals

et al. 1997; Shah et al. 1997). In the *npr1* mutants, systemic induction of *PR* genes and resistance is abolished. The characterization of NPR1 showed that it is a nuclear-localized protein (Kinkema et al. 2000), which may be involved directly in the regulation of *PR* genes through physical interaction with the TGA subclass of bZIP transcription factors (Després et al. 2000; Zhang et al. 1999; Zhou et al. 2000).

NPR1 is the only signaling component known to function downstream of SA. The *npr1* mutant has been used widely to define the SA-dependent signaling pathway. These studies show that *npr1* blocks biological induction of *PR* genes and resistance in systemic tissues but only has a minor effect on local gene induction and resistance (Clarke et al. 2000; Rogers and Ausubel 1997; Volko et al. 1998). A SA-dependent but NPR1-independent pathway is believed to be involved in local resistance induced after pathogen infection. Ethylene and jasmonic acid are implicated as signals that can induce NPR1-independent *PR* gene expression and pathogen resistance (Bowling et al. 1997; Clarke et al. 1998, Clarke et al. 2000; Epple et al. 1995; Penninckx et al. 1996; Penninckx et al. 1998).

To identify new signaling components in the NPR1-dependent and -independent pathways leading to *PR* gene expression and pathogen resistance, a genetic screen for *npr1* suppressors was performed. This screen resulted in the identification of the *sni1* (suppressor of *npr1-1*, inducible 1) mutant and cloning of the *SNI1* gene (Li et al. 1999). In the *npr1* mutant background, the recessive *sni1* mutation restored inducible *PR* gene expression and resistance in systemic tissues, whereas in the wild-type *NPR1* background, *sni1* caused enhanced sensitivity to SA and INA (Li et al. 1999; R. Mosher and X. Dong, *unpublished data*). These data showed that SNI1 is a negative regulator of SAR, which may function downstream of NPR1.

From the same suppressor screen that led to the isolation of *sni1*, the *snc1* (suppressor of *npr1-1*, constitutive 1) mutant was obtained. Here, we report the characterization of *snc1*, which displays constitutively high levels of *PR* gene expression as well as shows constitutive resistance to a virulent bacterial pathogen *Pseudomonas syringae* pv. *maculicola* ES4326 (*P. syringae maculicola* ES4326) and an oomycete pathogen *Peronospora parasitica* isolate Noco2 (*P. parasitica* Noco2) in the *npr1-1* background. The *snc1* mutation was mapped to a region on chromosome IV, which contains a cluster of *R* genes homologous to *RPP5*. The resistance conferred by the dominant *snc1* mutation was shown to be blocked by inactivating the *EDS1* gene, which is required for resistance mediated by certain members of the TIR-NB-LRR class R proteins, including RPP5, or by removing SA through expression of the *nahG* gene. This study demonstrates that the *snc1* mutation activates an EDS1-mediated *R* gene-signaling pathway, which is NPR1 independent yet SA dependent.

RESULTS

Identification of the *snc1* mutant.

We used the SA/INA-responsive *BGL2*::*GUS* reporter gene to originally identify the *npr1-1* mutant through its loss of inducible reporter gene expression (Cao et al. 1994). To identify new components in the NPR1-dependent and -independent resistance pathways, we looked for *npr1-1* suppressors by screening for constitutive and inducible *BGL2*::*GUS* expression in the *npr1-1* background. The characterization of the *sni1* suppressor mutant, which has inducible *BGL2*::*GUS* expression, was reported previously (Li et al. 1999). We also screened for *npr1-1* suppressors with constitutive expression of *BGL2*::*GUS*. Among the 7,000 EMS-mutagenized M_2 progeny screened, 12 showed constitutive *BGL2*::*GUS* reporter gene expression and were named *snc* (suppressor of *npr1-1*, constitutive). In addition to constitutive *PR* gene expression, the *snc1* mutant exhibited significantly enhanced resistance to pathogens. Therefore, we focused on the characterization of this mutant.

The *snc1 npr1-1* plants are small, have curly leaves, and show yellowing in the center of the rosette (Fig. 1). These morphological phenotypes are typical of plants with high levels of SA (Bowling et al. 1997). The inspection of *snc1 npr1-1* and *snc1 NPR1* mutants showed no macroscopic lesions. Trypan blue staining of these mutants further confirmed that the *snc1* mutation does not lead to lesion formation (Fig. 1).

When *snc1 npr1-1* (*BGL2*::*GUS*) was backcrossed with *SNC1 npr1-1* (*BGL2*::*GUS*), the F_1 progeny expressed the *BGL2*::*GUS* reporter gene, indicating that *snc1* is a dominant or haplo-insufficient mutation. Among the 135 F_2 progeny, 101 had beta-glucuronidase (GUS) staining, which further confirmed that this *snc1* phenotype is caused by a single dominant mutation (expected ratio: 3:1, $\chi^2 = 0.0006$; $P > 0.9$). Interestingly, whereas the constitutive expression of *BGL2*::*GUS* observed in *snc1* is a dominant trait, the morphological phenotype of *snc1* seems to be a recessive trait. The *snc1* heterozygous plants are wild type in morphology. When *snc1 npr1-1* (*BGL2*::*GUS*) was crossed with the wild type (*BGL2*::*GUS*), all F_1 plants constitutively expressed *BGL2*::*GUS*, which was expected for a dominant mutation. The F_2 progeny of this cross were then grown in the presence of INA to determine the segregation pattern of *snc1* in relationship to *npr1-1*. Of the 62 plants examined, 51 showed GUS staining, indicating that *snc1* segregated independently of *npr1-1* (expected ratio: 15:1, $\chi^2 = 0.84$; $P > 0.3$). Thus, we conclude that *snc1* is a second-site mutation unlinked to *npr1-1* and the expression of the *snc1* phenotype is independent of *npr1-1*.

PR gene expression and resistance in the *snc1* mutant.

Expression of *BGL2*::*GUS* in the *snc1* mutant is activated through an NPR1-independent pathway. In the *snc1* mutant, GUS staining was detected mainly along the petioles and veins, whereas in the wild type, SA- or INA-induced GUS staining was concentrated in the leaves but not in the petioles (data not shown).

In addition to the expression of the reporter gene, we examined the expression of *PR-1* in *snc1* by RNA gel blot analysis. The *snc1* mutant plants expressed the *PR-1* gene constitutively (Fig. 2), with overall expression levels higher in the wild-type *NPR1* background than in the *npr1-1* mutant background. Induction of *PR-1* expression by INA was observed in *NPR1*, not in *npr1-1*. The fact that *snc1* expresses *PR-1* to a higher level in the *NPR1* background than in the *npr1-1* mutant suggests that *snc1* also may activate the *NPR1*-dependent pathway.

snc1 npr1-1 and *snc1 NPR1* plants were infected with the virulent bacterial pathogen *P. syringae maculicola* ES4326 and the oomycete pathogen *P. parasitica* Noco2. Both *snc1 npr1-1* and *snc1 NPR1* mutants showed resistance to these

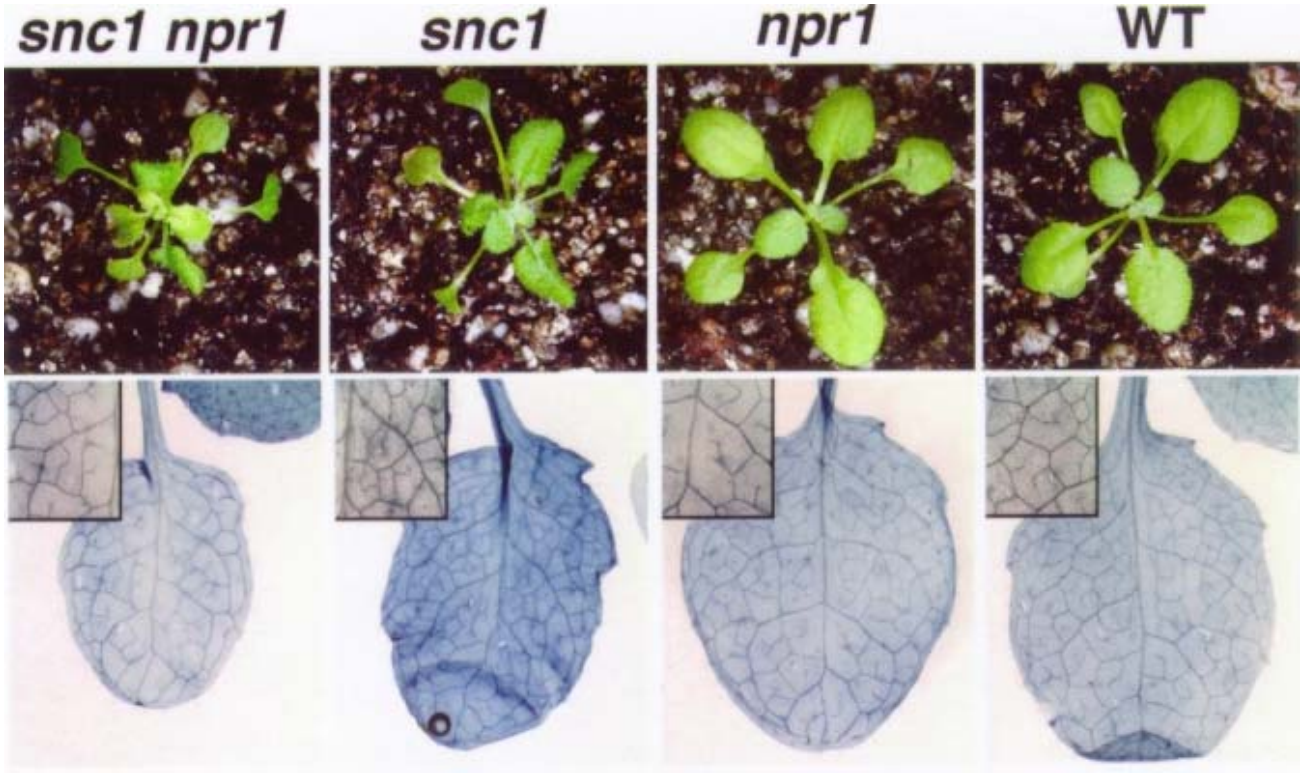

Fig. 1. Morphology and Trypan blue staining of wild-type (WT), *npr1*, *snc1*, and *snc1 npr1* plants. Plants were grown on soil for 3 weeks before the photographs were taken (left panels). Trypan blue staining was then performed according to Bowling et al. (1997) to detect possible necrotic lesions (right panels). Insets show close-up views of the Trypan blue-stained leaves.

two pathogens, even without INA induction (Fig. 3A and B). A further reduction in *P. syringae maculicola* ES4326 growth was detected in *snc1 NPR1* compared with *snc1 npr1* (Fig. 3A), indicating that *snc1* activates NPR1-independent and -dependent resistance to this pathogen. Interestingly, the resistance observed in the *snc1* plants is not associated with HR, as shown by the absence of Trypan blue staining after infection by *P. parasitica* Noco2 (Fig. 3C).

SA levels in the *snc1* mutant.

snc1 morphology commonly is seen in plants with high levels of SA (Bowling et al. 1997), suggesting that the endogenous SA levels in *snc1* are elevated. High-pressure liquid chromatography (HPLC) was used to measure SA levels in the *snc1* mutant and wild-type plants. We found that *snc1 NPR1* accumulated 15× more SA than the wild-type control (Fig. 4). Remarkably, in the *snc1 npr1-1* double mutant, the SA level was 21-fold higher than in the *snc1* single mutant. This is consistent with our hypothesis that NPR1 is involved in SA signaling as well as feedback regulation of SA once SAR is induced (Clarke et al. 2000; W. Fan and X. Dong, *unpublished data*).

A cross between the *snc1 npr1-1* mutant (*BGL2*::*GUS*) and a *nahG* transgenic line (without *BGL2*::*GUS*) was performed to determine whether SA is required for the *snc1* phenotype. In the F_1 progeny, the constitutive *BGL2*::*GUS* expression observed in *snc1* was suppressed by *nahG*, and no plants showed any GUS staining (data not shown). This indicates that SA is required for the *snc1*-induced expression of *BGL2*::*GUS*. In the F_2 progeny, there were four genetic loci segregating: *snc1*, *npr1-1*, *nahG*, and *BGL2*::*GUS*. Among the 89 F_2 plants examined, only 10 had GUS staining. This ratio supports the hypothesis that *nahG* suppresses the *snc1* phenotype (expected ratio: 9:55, $\chi^2 = 0.26$; $P > 0.6$; see below for derivation of the expected ratio).

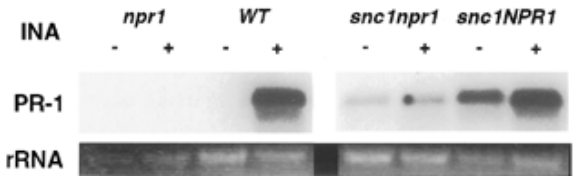

Fig. 2. *PR-1* gene expression in wild-type (WT), *npr1*, *snc1 npr1*, and *snc1 NPR1* plants. RNA was prepared from 2-week-old seedlings grown on MS media (Murashige and Skoog 1962), with or without 0.1 mM 2,6-dichloroisonicotinic acid (INA). Then, 10 μg of total RNA was loaded. The probe for *PR-1* was made according to Bowling et al. (1997). The 18S rRNA band detected by ethidium bromide staining was used as a loading control.

Mapping of the *snc1* mutation.

To map the *snc1* mutation *snc1 npr1-1* (in the *Arabidopsis thaliana* cv. Columbia ecotype, carrying *BGL2*::*GUS*) was crossed with the wild type (in the polymorphic Landsberg ecotype, also carrying *BGL2*::*GUS*) to generate a segregating population. In the F_2 progeny, plants homozygous at the *snc1* locus were identified on the basis of recessive morphological phenotype of *snc1*. A total of 792 *snc1* homozygotes were collected. Homozygosity at the *snc1* locus was confirmed in the F_3 generation by the expression of *BGL2*::*GUS* by all progeny.

Codominant cleaved amplified polymorphic sequences (CAPS) markers (Konieczny and Ausubel 1993) were used to map *snc1* to chromosome IV between SC5 (approximately 1.0 cM) and g4539 (approximately 0.2 cM) (Fig. 5). Additional CAPS markers were generated on the basis of the genomic sequence around this region (Bevan et al. 1998). The interval that contains *snc1* was delimited further to a 120-kb region with CAPS markers 13-*Eco*RV and 20-*Mbo*II. This 120-kb region covers the *RPP5 R*-gene cluster (Parker et al. 1997).

Epistasis analysis between the *snc1* mutant and two loss-of-resistance mutants, *eds1* and *ndr1*.

If *snc1* constitutively activates an *R* gene resistance pathway, the expression of the *snc1* phenotype may require either EDS1 or NDR1. In Arabidopsis, EDS1 has been shown to be required for the signaling of many TIR-NBS-LRR class R proteins, including RPP5, whereas NDR1 is required for the function of many LZ-NBS-LRR class R proteins (Aarts et al. 1998). Epistasis analysis was performed between *snc1* and

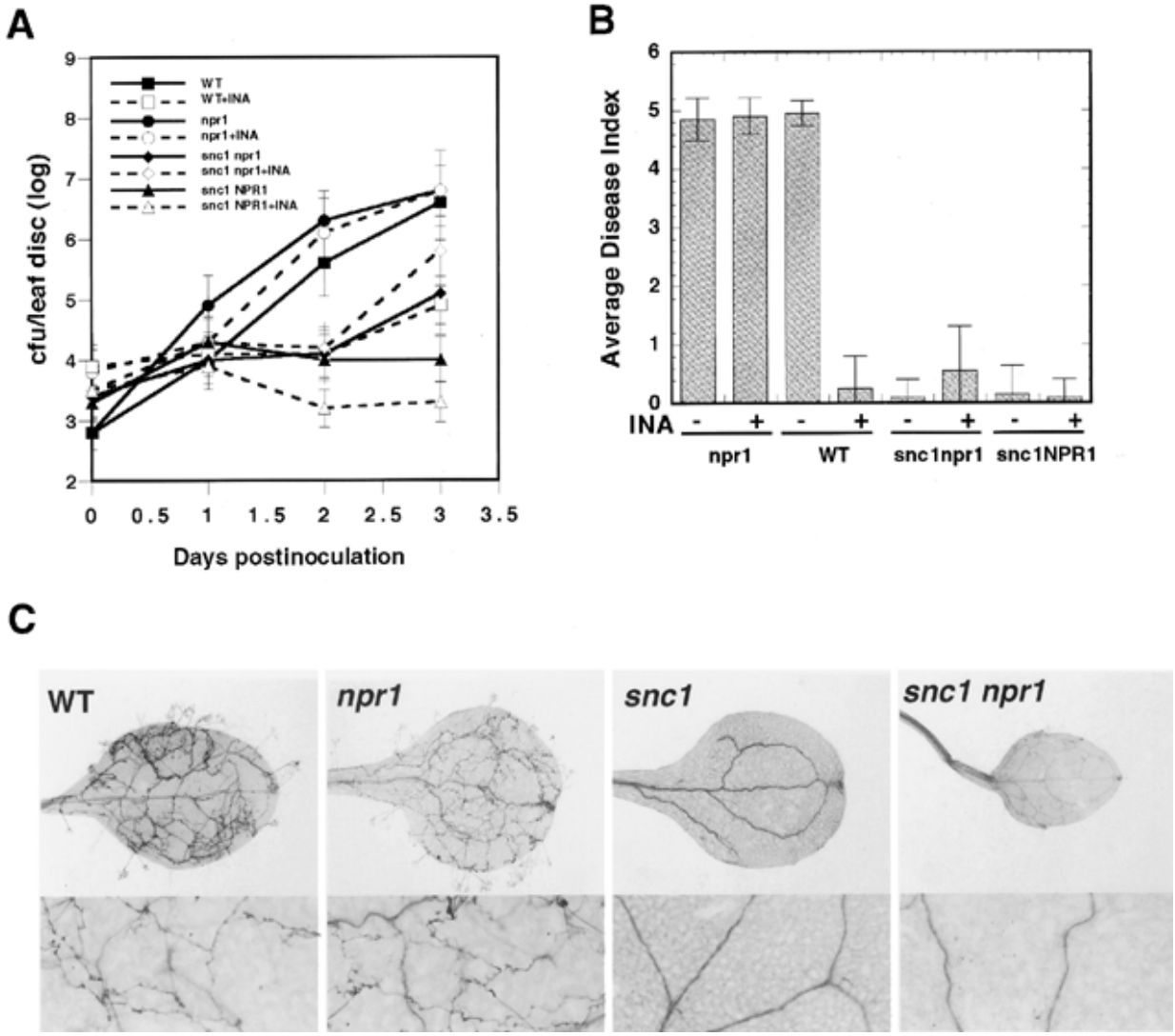

Fig. 3. Growth of *Pseudomonas parasitica* Noco2 and *Pseudomonas syringae maculicola* ES4326 in wild-type (WT), *npr1*, *snc1 npr1*, and *snc1 NPR1* plants. **A,** Growth of *P. syringae maculicola* ES4326. For *P. syringae maculicola* ES4326 infection, 4-week-old soil-grown plants were treated with 0.65 mM 2,6-dichloroisonicotinic acid (INA) 2 days prior to infection. Plants were infected by dipping into a *P. syringae maculicola* ES4326 suspension (optical density at 600 nm at 0.2) in 10 mM $MgCl_2$ and 0.01% silwet L-77 (Bowling et al. 1994). Eight leaves were excised for each genotype, treatment, and time point (0, 1, 2, and 3 days) and rinsed with H_2O and weighed, and bacteria was extracted and plated according to Bowling et al. (1994). Error bars represent 95% confidence limits of log-transformed data from four replicates (Sokal and Rohlf 1981). cfu = colony-forming units. **B,** Growth of *P. parasitica* Noco2. For *P. parasitica* Noco2 infection, 2-week-old seedlings were treated with INA (0.65 mM) 2 days prior to infection with a spore suspension (3×10^4 spores per ml). Disease symptoms were scored 7 days after infection by counting the number of conidiophores on each seedling (30 seedlings were examined for each phenotype and treatment). 0 = no conidiophores on the plants; 1 = no more than five conidiophores per infected leaf; 2 = 6 to 20 conidiophores on a few of the infected leaves; 3 = 6 to 20 conidiophores on most of the infected leaves; 4 = five or more conidiophores on all infected leaves; 5 = 20 or more conidiophores on all infected leaves. Average disease ratings with standard errors were calculated from 30 seedlings. **C,** Trypan blue staining. At 7 days after infection by *P. parasitica* Noco2, seedlings were stained with Trypan blue (Bowling et al. 1997) to detect hyphal growth and plant cell death.

eds1 or *ndr1* by constructing double mutants *snc1 eds1* and *snc1 ndr1*. The homozygous *snc1 eds1* double mutant lost the *snc1* morphological trait, expression of the *BGL2*::*GUS* reporter gene, and constitutive resistance to *P. syringae maculicola* ES4326 and *P. parasitica* Noco2 (Fig. 6). The homozygous *snc1 ndr1* double mutant, however, was similar to *snc1* in morphology and resistance to the two pathogens. These data suggest that *eds1* is epistatic to *snc1* and that the expression of the *snc1* mutant phenotype requires the function of EDS1 but not NDR1.

DISCUSSION

Resistance in *snc1* is NPR1 and cell death independent.

The *snc1* mutant shows constitutive *PR* gene expression and pathogen resistance in the *npr1-1* background. The level of resistance to *P. syringae maculicola* ES4326 and *P. parasitica* Noco2 that was observed in *snc1 npr1* is similar to that of an INA-treated wild-type plant. This suggests that *snc1* activates a NPR1-independent pathway that is highly effective against both pathogens. NPR1-independent resistance also has been observed in the *cpr5* and *cpr6* mutants, which were identified by their constitutive *PR* gene expression and resistance. Interestingly, in the *cpr5 npr1* and *cpr6 npr1* double mutants, resistance to *P. syringae maculicola* ES4326 is diminished, whereas resistance to *P. parasitica* Noco2 is not affected by *npr1* (Bowling et al. 1997; Clarke et al. 1998; Clarke et al. 2000). This suggests that the resistance pathway activated by *snc1* may be different from that activated in *cpr5* and *cpr6*. NPR1-independent gene expression and resistance also were studied in the lesion-mimic mutants *ssi1* and *acd6* (Rate et al. 1999; Shah et al. 1999). Resistance to *P. syringae maculicola* ES4326 in the *acd6* mutant was shown to be compromised only partially by *npr1*. These mutants form spontaneous lesions, and it is unclear to what extent cell death contributes to the NPR1-independent resistance observed in these mutants. Because *snc1* does not lead to spontaneous cell death under the conditions tested, it is unlikely that cell death is the trigger for the NPR1-independent resistance observed in *snc1*.

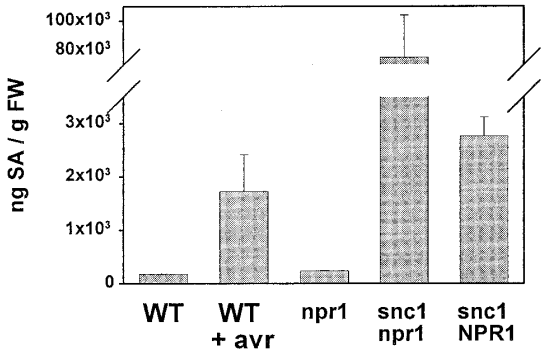

Fig. 4. Salicylic acid (SA) levels in wild-type (WT), *npr1*, *snc1 npr1*, and *snc1 NPR1* plants. Leaves from 4-week-old, soil-grown plants were collected and analyzed with high-pressure liquid chromatography to measure the levels of free SA, as described by Li et al. (1999). Values are averages of three replicates ± standard deviation. +avr: Plants infected with *Pseudomonas syringae maculicola* ES4326/*avr*Rpt2 at 3 days prior to tissue collection to induce SA production.

SA is essential for the NPR1-independent resistance in *snc1*.

In the *snc1* plants, SA levels are elevated. Interestingly, this phenotype is amplified in the *npr1* mutant background, indicating that NPR1 may be involved in feedback regulation of SA, as previously predicted (Clarke et al. 2000). Removal of SA from the *snc1 npr1* mutant plants through expression of *nahG* completely abolishes *PR* gene expression and pathogen resistance, demonstrating that SA is required for the expression of the *snc1* phenotype. SA alone, however, is not sufficient to bypass the *npr1* mutation because the application of SA to *npr1* plants does not lead to *PR* gene expression and pathogen resistance (Cao et al. 1994). In addition to SA, the *snc1* mutant must produce another signal that is required for induction of *PR* gene expression and pathogen resistance in the *npr1* background. Such a signal also may be produced in the *cpr* mutants which, like *snc1*, exhibit NPR1-independent pathogen resistance (Clarke et al. 2000). Similar observations were made for the lesion-mimic mutants *lsd6*, *lsd7*, *ssi1*, and *acd6* (Rate et al. 1999; Shah et al. 1999; Weymann et al. 1995). Whereas removal of SA abolishes the lesion-forming phenotype, the application of SA alone does not cause lesions in wild-type plants. Therefore, an additional signal must be produced by these mutants to initiate lesion formation.

snc1 activates a *RPP5*-related *R*-gene pathway.

The mapping of the *snc1* mutation to the *RPP5 R*-gene cluster (Noël et al. 1999; Parker et al. 1997) led us to speculate that a *RPP5* homolog may be activated constitutively in the *snc1* mutant. This activation may arise from a mutation in a *RPP5* homolog, which either activates or deregulates the R protein function. The notion that a *RPP5*-related *R*-gene pathway is activated in the *snc1* mutant was supported further by the epitasis analysis performed among the *snc1*, *eds1*, and *ndr1* mutants. In the *snc1 eds1* double mutant, the *snc1* mutant morphology, *PR*-gene expression, and pathogen resistance were blocked completely, whereas in the *snc1 ndr1* double mutant, only a minor effect was observed on the *snc1* mutant phenotype (Fig. 6). This is consistent with the fact that *snc1* mapped to the cluster of *RPP* homologs and the TIR-NB-LRR class of R proteins require EDS1 (Aarts et al. 1998) but not NDR1 as a downstream signaling component.

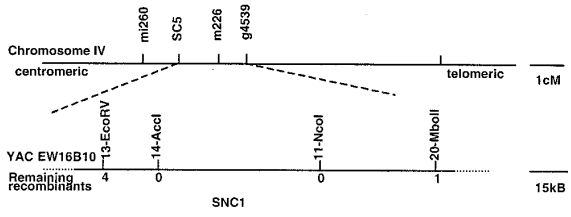

Fig. 5. Mapping of the *snc1* locus on chromosome IV. Codominant cleaved amplified polymorphic sequences (CAPS) markers (Konieczny and Ausubel 1993) were used to map *snc1* to chromosome IV between SC5 (approximately 1.0 cM) and g4539 (approximately 0.2 cM). Additional CAPS markers were generated on the basis of genomic sequence around this region (Bevan et al. 1998). The interval that contains *snc1* was narrowed further to a 120-kb region with CAPS markers 13-*Eco*RV and 20-*Mbo*II, which detected four and one recombinants, respectively. This 120-kb region covers the *RPP5 R*-gene cluster (Parker et al. 1997).

The mechanism by which *eds1* blocks the *snc1* mutant phenotype is unknown. EDS1 contains a consensus sequence found in lipases (Falk et al. 1999), suggesting that EDS1 is required for hydrolysis of a lipid substrate to form a signal molecule in *snc1*. The *eds1* mutation also abolishes the *snc1* morphological phenotype associated with high levels of SA, indicating that this signal molecule may be required for the accumulation of SA in *snc1*. In addition to SA, a signal must be produced in *snc1* to trigger the NPR1-independent resistance because SA alone is not sufficient to bypass the block of the *npr1* mutation. It is plausible that this unknown signal is produced through the activity of EDS1.

This study shows that a mutation in the *SNC1* gene leads to constitutive activation of an *R* gene-mediated resistance path-

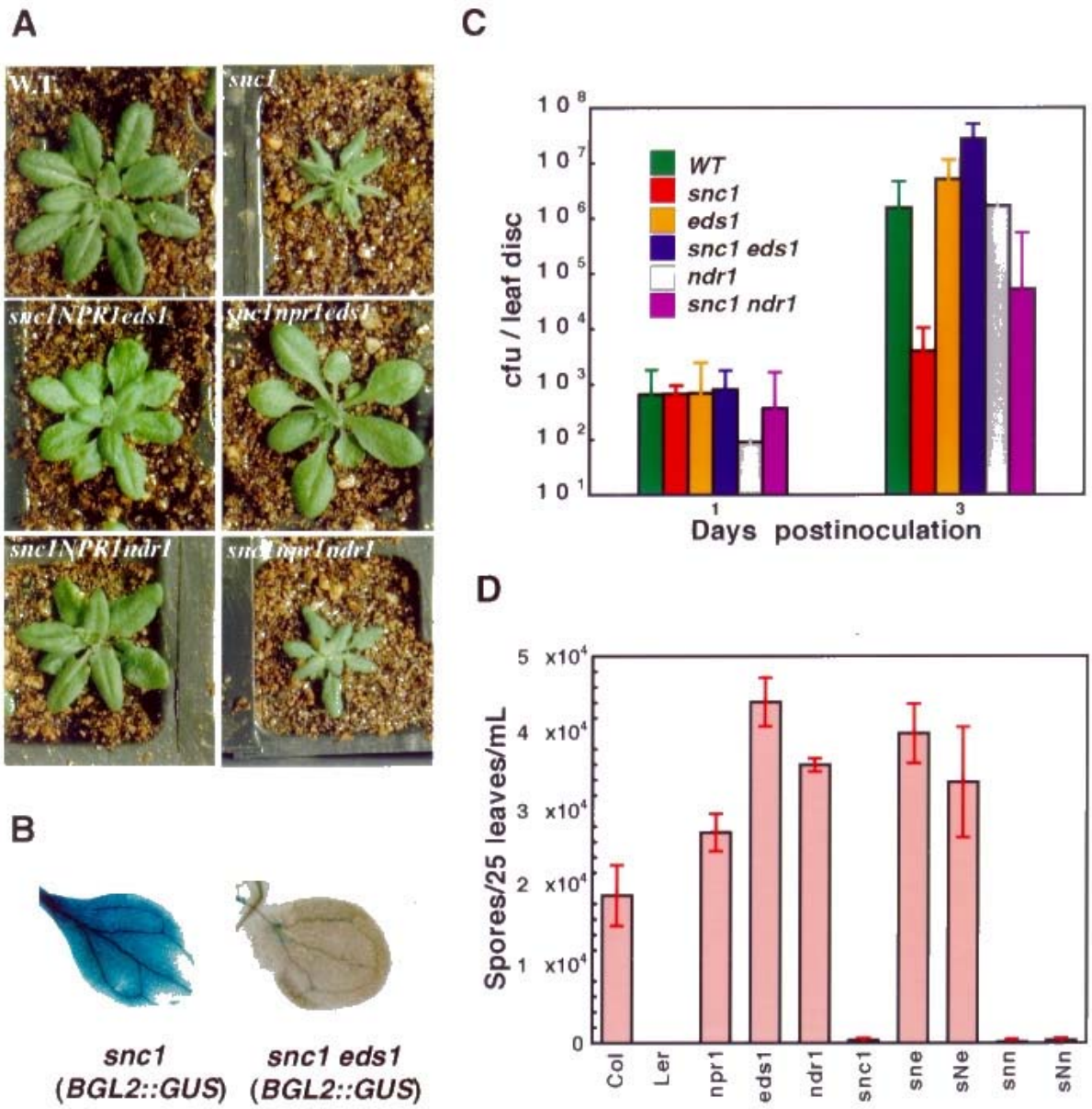

Fig. 6. Characterization of *snc1 eds1* and *snc1 ndr1*. **A,** Morphology of wild-type (WT), *snc1 eds1* (in *npr1* and *NPR1*), and *snc1 ndr1* (in *npr1* and *NPR1*) plants. Photographs were taken of 4-week-old soil-grown plants. **B,** Suppression of the *snc1*-induced reporter *BGL2*::*GUS* expression by *eds1*. Two-week-old seedlings grown on MS media (Murashige and Skoog 1962) were stained for beta-glucuronidase (GUS) activity according to Bowling et al. (1994). **C,** Suppression of the *snc1*-induced resistance to *Pseudomonas syringae maculicola* ES4326 by *eds1*. WT, *snc1*, *eds1*, *ndr1*, *snc1 eds1*, and *snc1 ndr1* plants were infected by infiltrating a suspension of *P. syringae maculicola* ES4326 in 10 mM $MgCl_2$ corresponding to an optical density at 600 nm of 0.001. Leaf discs were collected immediately following (day 0) and 3 days after infection (day 3). Four samples from each genotype were collected on day 0, and six samples were collected on day 3. Error bars represent 95% confidence limits of log transformed data (Sokal and Rohlf 1981). **D,** Suppression of the *snc1*-induced resistance to *Pseudomonas parasitica* Noco2 by *eds1*. *P. parasitica* Noco2 infection was accomplished by spraying a conidiospore suspension (3×10^4 spores per ml) onto 2-week-old plants. The infection was quantified 7 days later with a hemacytometer to count a 10-µl aliquot of spores harvested from 25 leaves in 1 ml of water. Two independent counts from each sample were averaged. Averages from three independent samples were used to calculate the number of spores per 25 leaves per ml ± standard deviation. Col, *Arabidopsis thaliana* cv. Columbia ecotype in which *snc1* and *ndr1* were isolated. L*er*, Landsberg ecotype in which *eds1* was identified. sne, *snc1 npr1 eds1*; sNe, *snc1 NPR1 eds1*; snn, *snc1 npr1 ndr1*; and sNn, *snc1 NPR1 ndr1*.

way (Fig. 7). This pathway requires the function of EDS1 and the signal molecule SA. Moreover, in *snc1*, resistance is conferred in the absence of a typical HR. Further characterization of *snc1* and *snc1*-like mutants will provide new information on the mechanism of *R* gene-mediated resistance.

MATERIALS AND METHODS

Mutant screen, characterization, and genetic analysis.

The screen for suppressors of *npr1-1* was carried out as described previously (Li et al. 1999). The EMS mutagenized M_2 plants, which are homozygous at the *npr1-1* locus and carry the *BGL2*::*GUS* reporter gene, were grown on MS medium (Murashige and Skoog 1962) containing 0.1 mM INA. The expression of the *BGL2*::*GUS* reporter gene was measured on 2-week-old seedlings with use of a protocol described previously (Bowling et al. 1994). Seedlings that showed GUS activity were transplanted to soil and allowed to set seeds. *BGL2*::*GUS* expression was retested on the collected mutants, with and without INA induction. Mutants that showed GUS activity, even in the absence of INA, were categorized as *snc* mutants.

The Mendelian character of the *snc* mutants were determined by backcrossing them with the parental line, *SNC npr1-1*. F_1 and F_2 progeny were examined for *BGL2*::*GUS* expression and morphology. To determine whether a *snc* mutation is linked to *npr1-1*, the *snc* mutant *snc npr1-1* was crossed with the wild type carrying *BGL2*::*GUS*. The segregation of *snc* and *npr1-1* mutations was examined in the F_2 progeny by scoring for INA-induced *BGL2*::*GUS* expression. From the segregating population, the *snc* single mutants, which are wild type at the *NPR1* locus, were identified with the morphological character of *snc1* and a restriction polymorphism between *NPR1* and *npr1-1* (Cao et al. 1997).

RNA gel blot analysis was used to measure *PR* gene expression in the *snc* mutants, as described previously (Cao et al. 1994). Infection by *P. syringae maculicola* ES4326 and *P. parasitica* Noco2 was conducted, and resistance was measured according to previously described procedures (Bowling et al. 1997; Clarke et al. 2000). HPLC, following a protocol derived from Raskin et al. (1989) and described in Li et al. (1999), was used to measure the endogenous SA levels in the mutants.

Mapping of *snc1*.

To map the *snc1* mutation, *snc1 npr1-1* in the Columbia ecotype (Col) was crossed with the wild type in Landsberg ecotype (L*er*). The F_2 progeny homozygous for *snc1* were identified according to the morphological phenotype of *snc1*. For crude mapping, 66 F_2 progeny homozygous for *snc1* were tested with a set of CAPS markers covering all five chromosomes of Arabidopsis (Konieczny and Ausubel 1993). When the marker AG was used, four recombinants were found, whereas when SC5 was used, one recombinant was discovered. The recombinants found for the two markers are exclusive, indicating that *snc1* is located on chromosome IV between AG and SC5. A total of 792 progeny homozygous for *snc1* were then collected, and the location of the *snc1* locus was narrowed to between SC5 and g4539, with thirteen and three exclusive recombinants found with these markers, respectively. Because the region between SC5 and g4539 was sequenced completely, more CAPS markers were generated according to sequence information. Marker 13 was amplified with primers 5′-CCGAGTAACGTTCTTTTCC-3′ and 5′-GGCTTTACTCCGCCGTG-3′, and the polymorphism between Col and L*er* was detected after restriction digestion with *Eco*RV. Marker 20 was amplified with primers 5′-CTTCACTGCCTCGTAG-3′ and 5′-GCATAAGCAGGTATG-CAG-3′, and polymorphism was revealed after digestion with *Mbo*II. Marker 14 was amplified with primers 5′-GTGTACAGAGGCTTACGC-3′ and 5′-GAAGTCTGGTTCCAGGAG-3′, and the fragment was digested with *Acc*I to detect the polymorphism. Marker 11 was amplified with primers 5′-GATCGGAAAGCTGTGCAG-3′ and 5′-GACATGCGAGGACAAAG-3′, and polymorphism was revealed after *Nco*I digestion. Markers 13-*Eco*RV and 20-*Mbo*II flanked the *snc1* mutation with four and one identified exclusive recombinants, respectively.

Epistasis analysis of *snc1*.

To determine whether SA is required for the *snc1* phenotype, the *snc1 npr1-1* mutant (with the *BGL2*::*GUS* reporter gene) was crossed with *nahG* (without the reporter) (Bowling et al. 1994). In the F_2 population of this cross, there were four

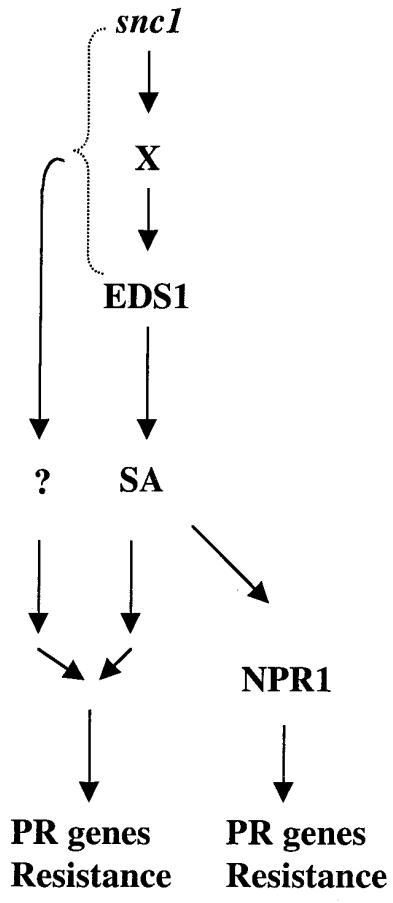

Fig. 7. Working model for *snc1*-induced resistance. The dominant *snc1* mutation constitutively activates an *R*-gene-mediated resistance pathway through an unknown factor (X) and EDS1, which is proposed to be required for the accumulation of salicylic acid (SA). In addition to SA, an unidentified signal (?) also is produced in *snc1* to induce NPR1-independent resistance.

genetic loci segregating: *snc1*, *npr1-1*, *nahG*, and *BGL2*::*GUS*. The expression of *BGL2*::*GUS* was examined with GUS staining, as described previously (Bowling et al. 1994). Because *snc1* suppresses *npr1-1*, the presence of *npr1-1* should not affect the expression of *BGL2::GUS* in the segregating progeny. In the F_2 population, 3/4 of the plants carry at least one copy of the reporter, among which 3/4 will express *BGL2::GUS* because of the dominant *snc1* mutation. If SA is required for the *snc1* phenotype, then the GUS-positive population should decrease from 9/16 (9:7) to 9/64 (9:55).

The *snc1 eds1* and *snc1 ndr1* mutants were generated by crossing *snc1 npr1* with the *eds1-2* or *ndr1-1* homozygous mutant (Century et al. 1995; Parker et al. 1996). In the F_2 progeny, the double mutants were identified by polymerase chain reaction (PCR) with primers flanking the deletions in *ndr1-1* and *eds1-2* (Aarts et al. 1998; Century et al. 1997). The primers for *ndr1-1* were NDRF 5′-GACGAGATTGCTCATTGCCATTGG-3′ and NDRR 5′-TAGGCATGGTACAATAACCGGAACC-3′. The primers for *eds1-2* were EDS1F 5′-AGATCAATGGCGTTT-GAAGCTCTTA-3′ and EDS1R 5′-ACCCCATCATGAGACCATTTCAATC-3′. To detect the point mutation in *snc1*, PCR primers SNCF 5′-CTTCAATGGCGGTGTTTTC-3′ and SNCW 5′-GGCATGCGTAATCTGCAATATCTAA-3′ were used.

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