Novel Functions of Stomatatal Cytokinesis-Defective 1 (SCD1) in Innate Immune Responses against Bacteria

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Eukaryotes employ complex immune mechanisms for protection against microbial pathogens. Here, we identified SCD1 (Stomatatal Cytokinesis-Defective 1), previously implicated in growth and development through its role in cytokinesis and polarized cell expansion (Falbel, T. G., Koch, L. M., Nadeau, J. A., Segui-Simarro, J. M., Sak, F. D., and Bednarek, S. Y. (2003) Development 130, 4011–4024) as a novel component of innate immunity. In Arabidopsis, SCD1 is a unique gene encoding for the only protein containing a complete DENN (Differentially Expressed in Normal and Neoplastic cells) domain. The DENN domain is a largely uncharacterized tripartite protein motif conserved among eukaryotic proteins. We show that conditional scd1-1 plants containing a point mutation in a conserved DENN residue affected a subset of signaling responses to some bacterial pathogen-associated molecular patterns (PAMPs). Consistent with increased transcript accumulation of Pathogen-related (PR) genes, scd1-1 plants were more resistant to Pseudomonas syringae pathovar tomato (Pst) DC3000 infection implicating SCD1 as a negative regulator of basal resistance against bacteria. scd1-1 plants were different from known mutants exhibiting constitutive expression of PR (cpr)-like phenotypes, in that growth impairment of scd1-1 plants was genetically independent of constitutive immune response activation. For scd1-1, shift to elevated temperature or introduction of a mutant allele in Salicylic acid Induction-Deficient 2 (SID2) suppressed constitutive defense response activation. sid2-2 also repressed the resistance phenotype of scd1-1. Temperature shift and sid2-2, however, did not rescue conditional growth and sterility defects of scd1-1. These results implicate SCD1 in multiple cellular pathways, possibly by affecting different proteins. Overall, our studies identified a novel role for eukaryotic DENN proteins in immunity against bacteria.

In eukaryotes, the first line of immunity against invading microbial pathogens occurs when host cell surface receptors

3 The abbreviations used are: PAMP, pathogen-associated molecular pattern; SCD1, stomatal cytokinesis-defective 1; DENN, differentially expressed in normal and neoplastic cells; PR, pathogen-related; cpr, constitutive expression of PR; SA, salicylic acid; FLS2, flagellin-sensitive 2; PP22, active flg22; AF22, inactive flg22; LC-MS/MS, liquid chromatography-tandem mass spectrometry; WT, wild-type; qRT-PCR, quantitative real-time polymerase chain reaction; ROS, reactive oxygen species; MAPK, mitogen-activated protein kinase; Pst, P. syringae pathovar tomato; SID2, salicylic acid induction-deficient 2; CPN1/BON1, copine/bonzai; DAB, 3,3-diaminobenzidine; GST, glutathione S-transferase.

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temperature sensitivity (5). The molecular mechanism(s) leading to any defects in these scd1 mutant lines, however, are currently unknown.

Here, we show that in conditional scd1-1 plants, the DENN mutation affected SCD1 protein accumulation in a temperature-dependent manner. Making use of the less severe growth defective phenotype compared with scd1-2-null mutant plants and the temperature-sensitive nature of scd1-1 DENN (S131F), we provide evidence that in addition to its role in plant growth and the temperature-sensitive nature of scd1-1, and SCD1-393 (Table 1) and directly subjected to restriction digestion analysis (5) as template, subcloned into a modified pGEX4T-1 vector (16) and verified by sequencing. Bacterially-expressed GST-SCD1DENN fusion protein were solubilized from inclusion bodies and used for GST-SCD1DENN polyclonal antiserum production in rabbits using standard procedures. For affinity purification of SCD1DENN antibodies, serum was first cleared of GST-specific antibodies by incubating the serum overnight with GST cross-linked to Affi-Gel 10 according to the manufacturer’s instructions (Bio-Rad) and subsequently strip affinity-purified against solubilized GST-SCD1DENN fusion protein resolved on preparative SDS-PAGE and transferred onto nitrocellulose membranes.

**Immunoblot Analysis**—Immunoblot analysis of total proteins was done as described (13) using antibody concentrations: αSCD1, 1:1200; αFLS2, 1:3000; αMPK6, 1:3000; and αcalnexin (1:3000); ophospho–44/42 MAPK (α-P-MAPKact, Antibody #9101, 1:3000; Cell Signaling Tech., Danvers, MA).

**Apoplastic ROS Production and MAPK Activation**—Apoplastic ROS production and MAPK activation assays were performed as described (13) at indicated PAMP concentrations, times, and temperatures.

**Seedling Growth Inhibition and Callose Deposition**—Seedling growth inhibition and callose deposition assays were done as described (17) at indicated PAMP concentrations, times, and temperatures except in callose assays, seedlings were fixed and cleared in 95% ethanol.

**DAB Staining**—For detection of whole cell H2O2 (apoplastic and intracellular H2O2), DAB staining was done as described (18, 19) with the following modifications. Excised leaves of 4–5-week-old plants were vacuum infiltrated with 1 mg/ml DAB (3,5-diaminobenzidine; Sigma) and cleared by boiling in lactic acid/glycerol/EtOH (1:1:3) for 5–10 min.

**Bacterial Pathogen Assays**—Bacterial pathogen assays. LuxCDABE-tagged Pseudomonas syringae pv. tomato DC3000 (20) were grown for 3 days on King’s B Medium plates with appropriate antibiotics at 28–30 °C and resuspended in water to an OD of 0.005. Leaves of 4–5-week-old plants were syringe infiltrated with bacterial inoculums of 5 × 10^5 cfu/ml and left to dry. In bacterial plate assays, leaf discs were harvested at 0 and 3 dpi and ground in 10 mM MgCl2. The cfu/cm^2 was determined by plating serial dilutions of individual leaf extracts. To monitor bacterial growth in planta, luciferase signal from bioluminescent Pst DC3000 was
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detected in whole plants using a Photek HRPCS4 photon detection camera (21).

Quantitative Real-time PCR (qRT-PCR) Analysis—For elicited leaf samples, indicated PAMP concentrations were leaf syringe-infiltrated for indicated times. Seedlings (9-day-old) or leaf disks (from 4–5-week-old plants) were frozen in liquid nitrogen. Total RNA was extracted from frozen tissues and subjected to qRT-PCR reaction as described (22) with primers listed in Table 1. The expression of At2g28390 (SAND family protein gene) was used to normalize all qRT-PCR results because of its highly stable expression during defense responses (22).

Statistical Analysis—Statistical analysis was performed using unpaired two sample t tests.

RESULTS

In plants, the bacterial PAMP flagellin or its active peptide derivative flg22 (PF22) is perceived by Flagellin-Sensitive 2 (FLS2) (1, 2). Previously, we employed a large-scale co-immunoprecipitation strategy using an αFLS2 antibody to discover novel PF22-dependent signaling component(s) required for innate immunity against bacteria (13). Here, we used the same approach to identify a protein with an apparent molecular mass of about 140 kDa that immunoprecipitated with the αFS2 antibody in a PF22-independent manner from solubilized microsomal membranes of La-er cell culture (supplemental Fig. S1). Based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, this protein was identified as SCD1 (supplemental Table S1), a DENN-domain containing protein required for growth and development (5). Probing αFLS2 immunoprecipitates with a purified polyclonal antibody made against the SCD1 DENN domain provided additional support that the identified 140 kDa protein was SCD1 (supplemental Fig. S2). Recent studies identified proteins with dual roles in development and innate immunity (1, 13, 23, 24).

We therefore were interested in whether in addition to growth and development, SCD1 has role(s) in innate immunity, a function currently not ascribed to any eukaryotic DENN protein.

scd1-1 Is a Conditional Temperature-sensitive, Partial Loss-of-function Mutation—Prior to testing SCD1 function in innate immunity, we investigated whether we could exploit the less severe growth defects and the temperature-sensitive nature of scd1-1 relative to scd1-2 (Fig. 1, A and B) (5) for subsequent assays. To gain a better understanding of the molecular mechanism(s) leading to the differences in phenotypic severity between these scd1 mutants (5), we compared SCD1 mRNA and protein levels between scd1-1 and scd1-2 grown at 22 °C. In scd1-2, no SCD1 mRNA (Fig. 1C) or SCD1 protein (Fig. 1D; see also supplemental Fig. S3) was detected using qRT-PCR or immunoblot analysis with the αSCD1 antibodies, respectively. In contrast, phenotypic defects in scd1-1 were not due to changes in SCD1 mRNA accumulation (Fig. 1C) (5), but correlated with changes in SCD1 protein levels compared with wild type (WT). Long exposure of immunoblots showed reduced but detectable levels of SCD1 protein in scd1-1 (Fig. 1D; see also supplemental Fig. S3). Thus, DENN integrity may be important for proper SCD1 stability or folding as recently suggested for RME-4, a DENN protein from Caenorhabditis elegans (11).

scd1-1 seedlings grown at non-permissive (22 °C) and transferred for 4 days to permissive (17 °C) temperature consistently accumulated more SCD1 protein relative to scd1-1 grown continuously at 22 °C, but did not reach levels found in WT (Fig. 1E). Increased SCD1 protein levels correlated with alleviation of phenotypic defects upon temperature shift to 17 °C (Fig. 1A) (5). We conclude that scd1-1 is a conditional temperature-sensitive, partial loss-of-function mutation whereas scd1-2 is a complete loss-of-function mutation.

FIGURE 1. SCD1 mRNA and protein accumulation in the temperature-sensitive mutant scd1-1 and the null mutant scd1-2. A, growth of scd1 mutants after temperature shift experiments from non-permissive (22 °C) to permissive (17 °C) temperatures, scd1-1 (s1-1), scd1-2 (s1-2), and their WT (Col0g1 and Col0, respectively) seedlings were grown on Murashige and Skoog plates containing 0.8% sucrose (MS plates) at 22 °C for 9 days. Seedlings were grown on MS plates for an additional 9 days at 17 °C (22 → 17 °C) or 22 °C (cont.). B, after growth on MS plates for 9 days, WT (Col0g1), scd1-1, and scd1-1 complemented with pSCD1::SCD1 (scd1-1/comp) seedlings were grown in soil for an additional 4 weeks. All growth was at 22 °C. C, SCD1 mRNA expression levels. Total RNA isolated from scd1 mutants and WT were used as templates for qRT-PCR using At2g28390 as a reference gene. Samples (n = 5–6) were analyzed for each plant line. Statistical (asterisks) and non-statistical (ns) significance are shown. * indicates S.E. D, SCD1 protein accumulation in scd1 mutants. Total proteins were subjected to immunoblot analysis using αSCD1-DENN or αMPK6 (loading control) antibodies. E, increase in SCD1 protein accumulation in scd1-1 mutants after temperature shift from 22 to 17 °C. 7-day-old seedlings grown at 22 °C were shifted for 4 days to 17 °C or kept at 22 °C. Total proteins were subjected to immunoblot analyses using αSCD1-DENN antibody. PonceauS staining and αMPK6 antibodies were used as loading controls. Unless stated otherwise, 9-day-old seedlings grown at 22 °C were used. All experiments were done three times. Bars, 1 cm.
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Conditional scd1-1 Show Impaired PAMP-induced ROS Responses—To investigate SCD1 function in innate immunity, we focused on scd1-1. In contrast to scd1-2 (5), scd1-1 grew sufficiently at 22 °C for experimental manipulation (Fig. 1B). First, we tested whether SCD1 has a genetic role in PF22-dependent signaling responses. As determined by luminol-based assays (17), PF22-elicted rapid and transient accumulation of apoplastic (extracellular) reactive oxygen species (ROS) in small leaf pieces of 4–5-week-old WT plants (Fig. 2, A and B). At 22 °C, PF22-elicted ROS production was significantly reduced in scd1-1 leaves compared with WT at each time point (Fig. 2, A and B; p ≤ 0.0013), indicating a role for SCD1 in PF22-induced oxidative burst. In control experiments, no ROS were produced in response to DMSO or inactive flg22 (AF22) in scd1-1 or WT (Fig. 2, A and B). scd1-1 plants also displayed significantly impaired ROS production in response to elf26 (Fig. 2B; p < 0.005), a bacterial PAMP structurally unrelated to PF22 and perceived by an independent PAMP receptor, EFR (25). Thus, SCD1 function was not restricted to PF22-elicited ROS production.

To confirm that impaired PF22-induced ROS production was caused by the DENN mutation in SCD1, scd1-1 plants were grown at 22 °C continuously (22 °C) at 22 °C. ROS production (scd1-1) were produced in response to DMSO or inactive flg22 (AF22) in PF22-induced oxidative burst. In control experiments, no ROS (Fig. 2, A and B). scd1-1 plants also displayed significantly impaired ROS production in response to elf26 (Fig. 2B; p < 0.005), a bacterial PAMP structurally unrelated to PF22 and perceived by an independent PAMP receptor, EFR (25). Thus, SCD1 function was not restricted to PF22-elicited ROS production.

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were increased after PF22 elicitation compared with mock-treated tissue. WT and scd1-1 plants showed no statistically significant differences in induction of these marker transcripts (Fig. 3B). Similar results were obtained from PF22-treated 9-day-old seedlings. We also investigated transcript accumulation of pathogen-related (PR) PR1, PR2, and PR5 genes, known late marker genes for PF22 signaling and defense responses (17, 27). Interestingly, PF22 elicitation for 24 h led to a significant increase in transcript accumulation of PR1, PR2, and PR5 genes in scd1-1 compared with WT leaves (Fig. 3C; p < 0.001). These results implicated SCD1 as a negative regulator of PF22-induced transcript accumulation of these marker PR genes.

Next, we determined whether PF22-dependent activation of mitogen-activated protein kinases (MAPKs) MPK3, MPK6, and MPK4, generally used to monitor early PAMP responses (2), was affected in scd1-1. After elicitation with increasing PF22 concentrations for 10 min, we did not observe any differences in the activation of these MAPKs when comparing scd1-1 and WT seedlings (Fig. 3D) using an antibody (α-P-MAPKact) that detects activated, phosphorylated MAPKs (13). Finally using a classical assay for late PAMP responses (17), we examined PF22-induced callose deposition in seedlings. PAMP elicitation induces synthesis of callose, a β-1,3-glucan polymer visualized as tiny bright dots by aniline blue staining (2, 17). After elicitation with PF22 for 24 h, scd1-1 seedlings showed callose deposition similar to WT (Fig. 3E) indicating that PF22-induced callose accumulation was independent of the SCD1 DENN mutation. In control experiments, no callose accumulation was observed in scd1-1 or WT seedlings in the absence of any treatment or in response to inactive flg22 (AF22; Fig. 3E). Taken together, SCD1 was required for some (ROS production, seedling growth inhibition, transcript accumulation of PR1, PR2, PR5 genes) but not other PF22-elicited signaling responses (transcript accumulation of tested WRKY genes, activation of MAPKs, callose deposition).

scd1 DENN Mutation Leads to Constitutive Activation of Defense Responses and Increased Basal Resistance Against Bacteria—During gene expression profiling of plant material grown at 22 °C, we observed increased transcript accumulation of PR genes in scd1-1 seedlings and leaves compared with WT in the absence of any PAMP elicitation (Fig. 3C; data not shown). PR1 is a defense marker gene for SA-regulated signaling responses whose expression is induced after PAMP treatment or pathogen infection (4, 28). Plant mutants showing constitutive expression of PR1 gene in the absence of any stimulus are categorized as cpr (constitutive expressor of PR) mutants (4). In general, cpr mutants exhibit constitutive activation of defense responses as exemplified by aberrant expression of hormone-inducible defense genes, activation of callose deposition and whole cell accumulation of H₂O₂ in the absence of any stimulus (18, 29–32).

Thus, we tested next whether non-treated scd1-1 displayed additional cpr-like phenotypes. We focused first on transcript accumulation of other hormone-inducible genes involved in defense responses using qRT-PCR analyses. Non-treated scd1-1 leaves accumulated statistically significant higher mRNA levels of the SA-marker genes PR1, PR2, and PR5 compared with WT (Figs. 3C and 4A; p ≈ 0.01). In contrast, mRNA levels of marker genes (LOX2 and EFR2) for jasmonic acid and ethylene, hormones with antagonistic immune response function to SA (28), were significantly reduced in scd1-1 compared with WT (Fig. 4A; p ≈ 0.01). Thus, scd1-1 showed aberrant regulation of hormone-inducible defense genes in the absence of any stimulus.

As described earlier, when examining callose deposition at the seedling stage (7–9-day-old), scd1-1, or WT did not display any significant callose accumulation in the absence of active PAMP treatment (see AF22; Fig. 3E). At a later stage of devel-
plants were syringe-infiltrated with 5 × 10^5 Pst DC3000 to eliminate any possible effect on bacterial growth due to the reduced number of functional stomata in scd1-1. Using bacterial plating assay, no statistical difference in bacterial growth (cfu/ml) was observed between scd1-1 and WT leaves at 0 dpi (Fig. 4D). But after 3 dpi, growth of Pst DC3000 was significantly reduced in scd1-1 leaves by more than 10-fold compared with WT (Fig. 4D; p < 0.0001; see also Fig. 5, C and D). These results implicated SCD1 genetically as a negative regulator of basal resistance against Pst DC3000. In control experiments, scd1-1 plants expressing the SCD1 gene under the control of its own promoter (scd1-1/comp) complemented bacterial growth repression at 3 dpi (Fig. 4D; p < 0.002).

**Growth and Developmental Impairments Can Be Uncoupled from Constitutive Activation of Defense Responses and Resistance Phenotypes in scd1-1**—In eukaryotes, growth and developmental defects have been attributed to constitutive activation of immune responses (3, 15). In cpr mutants, these defects correlate with up-regulation of SA-dependent signaling (i.e. increased PR1 transcript levels) and can be reversed genetically by introducing SID2 (Salicylic acid Induction-Deficient 2) mutant alleles impaired in defense-related SA biosynthesis (14, 28). To test whether the scd1-1 growth defect was SA-dependent, we generated homozygous scd1-1sid2-2 double mutants. In these double mutants, PR1 transcript accumulation was similar to WT levels indicating that sid2-2 reversed the increased accumulation of PR1 transcript present in scd1-1 (Fig. 5A, p = 0.0001). Furthermore, leaves of scd1-1sid2-2 plants did not display increased callose deposition observed in scd1-1 (Fig. 5B). Taken these data together (Fig. 5, A and B), sid2-2 suppressed constitutive activation of defense responses observed in scd1-1.

Next, we investigated whether inhibition of defense-related SA biosynthesis genetically suppressed the increased resistance against bacteria observed in scd1-1 plants (Figs. 4A, 5C, and 5D). To this end, scd1-1 single and scd1-1sid2-2 double mutant plants were syringe-infiltrated with a Pst DC3000 strain (5 × 10^5). WT (Colg1) and sid2-2 plants were used as controls. First, we determined changes in resistance phenotypes using bacterial plate assays (colony forming units (cfu)/cm²). No significant difference of bacterial growth was observed between plant lines at 0 dpi indicating equal infiltration (data not shown; Fig. 4D). At 3 dpi, scd1-1sid2-2 double mutant plants were significantly more susceptible to bacterial infection than scd1-1 and WT (Fig. 5C; p < 0.0001). More specifically, double mutant plants displayed increased bacterial growth to statistically similar levels as the sid2-2, a mutant known to have increased susceptibility to bacterial infection (14, 28). These results indicated that sid2-2 suppressed the resistance phenotype of scd1-1.

In our studies, plants were infiltrated with Pst DC3000 carrying a chromosome insertion of the luxCDABE operon, a bioluminescent bacterial strain whose luminescence has been reported to accurately and reliably reflect bacterial growth in infected Arabidopsis leaves (20, 21). Using this bioluminescent bacterial strain enabled us to monitor growth of bioluminescent Pst DC3000 in planta using a Photek luminescent camera system (Fig. 5D). Importantly, results obtained with this camera system were consistent with those from the bacterial plate assay.

FIGURE 4. Mature scd1-1 leaves showed constitutive activated defense responses and increased basal resistance against Pst DC3000 at 22 °C. A, aberrant hormone-inducible gene expression in non-treated scd1-1 (black bars) compared with WT (white bars). Leaf disks were processed for qRT-PCR with At2g28390 as a reference gene. Samples from 3– 4 independent experiments were analyzed for each plant line. B, increased callose deposition in untreated scd1-1 leaves. Leaves of non-treated plants were stained with aniline blue to visualize callose. Experiments were done twice for 3– 4 leaves taken from three different scd1-1 or WT plants. Bar, 0.5 mm. C, H₂O₂-stainable lesions in untreated scd1-1 leaves. Leaves of non-treated plants were stained with DAB to visualize H₂O₂-stainable lesions. Experiments were done twice for 3– 4 leaves taken from different scd1-1 or WT plants. Bar, 0.2 mm. D, increased bacterial resistance to Pst DC3000 in scd1-1. Leaves of scd1-1 (black bars), WT (white bars), and scd1-1/pSCD1::SCD1 (scd1-1/comp; gray bars) plants were syringe-infiltrated with 5 × 10^5 Pst DC3000. At 0 and 3 days post-infiltration (dpi), bacterial growth was assessed in leaf disks (n = 10–12) from individual leaves of three independent plants using bacterial plate assays. Experiments were performed 2–3 times. No statistical difference, ns; colony forming units per cm², cfu/cm². Asterisk indicates statistically significant differences. Plants were grown, and experiments were performed at 22 °C. * indicates S.E. WT is Colg1.
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Figs. 4D and 5C), in that (a) *scd1-1* plants showed reduced growth of luminescent bacteria compared with WT and (b) *sid2-2* showed increased bacterial growth compared with WT and *scd1-1* (Fig. 5D). Importantly, in agreement with plate assay results, *scd1-1sid2-2* plants supported increased bacterial growth compared with *scd1-1* and WT plants (Fig. 5D) indicating that *sid2-2* suppressed the resistance phenotype associated with *scd1-1*. Taken these results together (Fig. 5, A–D), we conclude that in *scd1-1*, both constitutive activation of defense responses and increased resistance against bacteria could be correlated genetically to defense-related SA biosynthesis.

We noted, however, that *sid2-2* was unable to rescue growth impairment of *scd1-1* because *scd1-1sid2-2* double mutants did not grow to larger size compared with *scd1-1* (Fig. 5E). Furthermore, *sid2-2* did not rescue the conditional flowering and sterility defects of *scd1-1* at 22 °C (Fig. 5F). Similar to the conditional, temperature-sensitive *scd1-1* (5), *scd1-1sid2-2* double mutant plants showed impairment in flower development and did not set seeds at 22 °C. Only after shift to permissive temperature (17 °C) did *scd1-1sid2-2* develop fertile flowers that produced siliques and seeds (Fig. 5G). Thus, in contrast to previously described mutants with *cpr* phenotypes (28), the growth and developmental defects in *scd1-1* plants were genetically independent (or upstream) of defense-related SA signaling pathway(s).

In *cpr*-like mutants, growth impairment and constitutive defense response activation can also be rescued by growing mutant plants at elevated temperatures as shown for plants with mutations in *coppine/bonzai* (*CPN1/BON1*) (15, 32). In agreement, temperature shift from 22 °C to 28 °C for 9 days rescued *bon1-1* growth impairment and repressed constitutive transcript accumulation of *PR1* gene compared with *bon1-1* continuously grown at 22 °C (Fig. 6, A and B). In *scd1-1*, shift to 28 °C also suppressed elevated *PR1* transcript accumulation (Fig. 6B, p ≤ 0.001). But in contrast to *bon1-1* and other mutants with *cpr*-like phenotypes (15, 18, 31, 34, 35), growth impairment of *scd1-1* was not rescued by shift to high temperature (Fig. 6A). These results provided additional evidence that growth and developmental defects of *scd1-1* could be uncoupled from constitutive activation of defense responses.

DISCUSSION

*SCD1* is implicated in plant growth and development, likely due to its role in cytokinesis and polarized cell expansion (5).
Here, we identified novel function(s) of SCD1 in innate immune responses against bacteria, some of which were independent of a SCD1 requirement in plant growth and development (Figs. 5 and 6). Thus, our study on SCD1 expands our limited knowledge on proteins with dual roles in innate immunity as well as growth and development (23, 24, 36).

We showed that the DENN mutation in SCD1 affected some (ROS production, seedling growth inhibition, PR transcript accumulation; Figs. 2 and 3), but not other (activation of gene transcription and MAPKs, callose deposition; Fig. 3) PF22-elicited signaling responses. The fact that in scd1-1, a subset of PF22-responses was induced to WT levels at the restrictive temperature (22 °C) suggested that FLS2-function (i.e. in PF22 perception) was not impaired in these mutant cells. Furthermore, FLS2 protein levels were similar in scd1-1 and WT plants independent of growth conditions (Fig. 2E and supplemental Fig. S3). These results indicate that in scd1-1, (a) the PF22-signaling defects at 22 °C were not due to overall reduced FLS2 receptor levels and (b) in temperature shift experiments to 17 °C, reversal of the ROS defect was not due to increased FLS2 protein levels. Consistent with the notion that impairment of some PF22 responses could not be attributed to decreased FLS2 receptor levels, FLS2 protein levels were also not reduced in scd1-2-null mutant seedlings (supplemental Fig. S3).

It is possible that the scd1-1 mutation may affect the function of PF22-signaling component(s) downstream of FLS2/PF22 perception. A potential candidate may be RbohD, the plant homolog of the mammalian gp91phox respiratory burst NADPH-oxidase subunit required for PF22-induced rapid burst of extracellular ROS (37, 38). The observed PAMP defects of scd1-1, however, cannot be solely explained by impaired RbohD function. In contrast to scd1-1, rbohD-null mutants are impaired in PF22-induced callose deposition (38) (data not shown). Furthermore, rbohD-null mutants do not exhibit any obvious growth defects or any resistance defect after bacterial infection (39). Thus, SCD1 may exert its PF22 signaling function via yet unknown component(s). Another possible explanation for why only a subset of PAMP responses was affected in scd1-1 may be the existence of parallel and/or multibranched PAMP signaling pathways rather than a single linear pathway (40).

In addition to functioning as a positive regulator of PAMP responses, our data also implicated SCD1 as a negative regulator of PF22 signaling responses. In scd1-1, PF22 elicitation led to increased transcript accumulation of PR1, PR2, and PR5 (Fig. 3C). Increased transcript accumulation of these SA-regulated defense marker genes may at least in part account for increased resistance against bacteria of scd1-1.

Furthermore, scd1-1 plants displayed constitutive activation of defense responses and increased resistance against Pst DC3000 (Fig. 4) implicating SCD1 genetically as a negative regulator of immune responses. These immune phenotypes are reminiscent of those described for cpr mutants. But in contrast to most previously described mutants with cpr-like phenotypes (15, 18, 31, 34, 35), SCD1 has a role in plant growth and development per se. Using two independent approaches (blocking SA biosynthesis genetically by introducing the sid2-2 mutant allele into scd1-1 and shift to high temperature; Figs. 5 and 6), we provide evidence that conditional growth and sterility defects of scd1-1 could be uncoupled from constitutive activation of defense responses and increased resistance to bacteria. We conclude that SCD1 appears to function in multiple and diverse cellular pathways.

It remains to be determined whether the role(s) of SCD1 as (a) a positive regulator of PAMP responses (ROS production, seedling growth inhibition), (b) a negative regulator of flg22-elicited PR transcript accumulation, and (c) a negative regulator of constitutive activation of defense responses and bacterial resistance are separate or interrelated functions. In support of the latter, recent studies (41, 42) suggest an interplay between PAMP-elicted and SA-mediated innate immune responses. Perhaps SCD1 may function in PAMP-induced activation of a yet unknown component that, in turn, may be required in attenuating SA-mediated defense responses. In this model, impaired PF22 activation of this component would lead to constitutive activation of defense responses and increased resistance against bacteria. This model is in agreement with a recent study (40) indicating that a significant element in mounting robust immunity involves sustained PAMP receptor signaling that induces and maintains transcriptional reprogramming at a relatively late phase (including that of PR genes).

We cannot, however, exclude the possibility that SCD1 may have independent and possibly antagonistic functions as shown for BAK1. BAK1 has dual roles as a positive regulator of PAMP responses (13, 24) and as a negative regulator of constitutive defense response activation (34). But in contrast to SCD1, BAK1 function in constitutive activation of defense responses is linked to its role in plant growth and development (34).

Interestingly, the mammalian DENN domain protein DENN/MADD has dual roles in neuronal processes as a positive and negative regulator, and these functions are exerted through targeting different proteins. Interaction of DENN/MADD with tumor necrosis factor receptor 1 (TNFR1) suppresses signaling pathways that regulate neuronal cell death (9). DENN/MADD also functions in neurotransmission by positively regulating small GTPase Rab3 activities (10, 43). Ulti-
Role of DENN Domain Protein in Innate Immunity

mately, reduced DENN/MADD levels impair overall growth and development (10, 44) and correlate with cell death and neurological diseases such as Alzheimer’s (9, 45). To our knowledge, however, animal DENN protein function in innate immune responses has not been reported. Gaining further insight into the roles of SCD1 in plant innate immunity and growth and development represents an exciting goal to advance our general understanding of DENN protein function in eukaryotes.

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