EDS5, an Essential Component of Salicylic Acid–Dependent Signaling for Disease Resistance in Arabidopsis, Is a Member of the MATE Transporter Family

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The eds5 mutant of Arabidopsis (earlier named sid1) was shown previously to accumulate very little salicylic acid and PR-1 transcript after pathogen inoculation and to be hypersusceptible to pathogens. We have isolated EDS5 by positional cloning and show that it encodes a protein with a predicted series of nine to 11 membrane-spanning domains and a coil domain at the N terminus. EDS5 is homologous with members of the MATE (multidrug and toxin extrusion) transporter family. EDS5 expression is very low in unstressed plants and strongly induced by pathogens and UV-C light. The transcript starts to accumulate 2 hr after inoculation of Arabidopsis with an avirulent strain of Pseudomonas syringae or UV-C light exposure, and it stays induced for ~2 days. EDS5 also is expressed after treatments with salicylic acid, indicating a possible positive feedback regulation. EDS5 expression after infection by certain pathogens as well as after UV-C light exposure depends on the pathogen response proteins EDS1, PAD4, and NDR1, indicating that the signal transduction pathways after UV-C light exposure and pathogen inoculation share common elements.

INTRODUCTION

Plants react to an attack by phytopathogenic microorganisms with an array of inducible defense responses. Whether a plant is resistant or susceptible to a potential pathogen depends largely on how fast a pathogen is recognized and defense responses are activated. For instance, in gene-for-gene resistance, the product of an avirulence gene of the pathogen is recognized by a corresponding resistance gene product of the plant, leading to the rapid activation of various defense responses. Such a pathogen is avirulent to the plant, its invasion can be stopped, and the plant is resistant. Disease ensues when the pathogen is not recognized rapidly and defense mechanisms are activated too slowly to stop the infection process. In this case, the pathogen is virulent and the plant is susceptible. In addition, defense responses can be induced systemically in all parts of the plant by pathogens, soil-borne microorganisms, chemicals, or certain forms of stress. This form of induced resistance is referred to as systemic acquired resistance (Ryals et al., 1994; Sticher et al., 1997).

Salicylic acid (SA) is synthesized after inoculation of plants with pathogens or exposure to certain abiotic stresses, such as ozone and UV-C light. SA was found to be essential for gene-for-gene resistance, systemic acquired resistance, and reduction of disease development after inoculation with virulent pathogens (Delaney et al., 1994; Nawrath and Métraux, 1999).

In Arabidopsis, the elucidation of the signal transduction pathway downstream of SA leading to the expression of a number of pathogenesis-related (PR) proteins, such as PR-1, PR-2, and PR-5, has been centered on the characterization of the npr1/nim1 mutant (Cao et al., 1994; Delaney et al., 1995). The npr1/nim1 mutant does not express PR-1, PR-2, and PR-5 after treatment with SA analogs, such as isonicotinic acid (Cao et al., 1994; Delaney et al., 1995). However, when infected with pathogens such as Pseudomonas syringae pv maculicola, only PR-1 expression is reduced strongly, indicating that pathogens may induce PR-2 and PR-5 in a NPR-independent manner (Glazebrook et al., 1996). The NPR1/NIM1 gene encodes a novel protein with ankyrin repeats (Cao et al., 1997; Ryals et al., 1997) that is translocated to the nucleus upon SA treatment (Kinkema et al., 2000). NPR1/NIM1 likely acts as a transcriptional coactivator that enhances the binding of basic leucine zipper protein transcription factors of the TGA family to the as1 element of the PR-1 promoter (Zhang et al., 1999; Zhou et al., 2000).

Several mutants of Arabidopsis have been isolated that are unable to establish defense responses (Glazebrook, 1999). NDR1, a small membrane-associated protein, is essential for the establishment of resistance after inoculation with certain avirulent pathogens, such as P. syringae carrying the avrRpt2 or avrRpm1 gene. In this case, effective activation of defense depends on resistance genes containing a
nucleotide binding site and a leucine-rich repeat domain (Century et al., 1995, 1997). EDS1, a protein with homology with lipases, is necessary for activation of the defense pathway after inoculation with virulent and certain avirulent pathogens, such as P. syringae carrying the avrRps4 gene (Parker et al., 1996; Aarts et al., 1998; Falk et al., 1999; Feys and Parker, 2000). PAD4 also contains a lipase domain (Glazebrook et al., 1997; Jirage et al., 1999) and is required for the activation of the SA-dependent defense pathway, the production of the phytoalexin camalexin, and the reduction of disease development after inoculation with virulent P. syringae and virulent as well as avirulent Peronospora parasitica isolates (Glazebrook et al., 1997; Zhou et al., 1998).

The eds5/sid1 (eds5 is allelic to sid1) (Rogers and Ausubel, 1997; Nawrath and Métrax, 1999) and sid2 mutants of Arabidopsis do not accumulate SA after inoculation with either virulent or avirulent pathogens or after abiotic stresses and demonstrate strongly reduced expression of PR-1. Both mutants display pathogen-induced increases in PR-2 and PR-5 expression, similar to that observed in the npr1/nim1 mutant (Glazebrook et al., 1996), and accumulate high levels of camalexin (Nawrath and Métrax, 1999; Dewdney et al., 2000). In contrast, SA-degrading NahG plants, despite endogenous SA levels as low as those in eds5 and sid2, show strong reduction in PR-1, PR-2, and PR-5 expression as well as reduced camalexin accumulation after pathogen inoculation (Delaney et al., 1995; Zhao and Last, 1996). The susceptibility to pathogens of the eds5 and sid2 mutants is intermediate between that of wild-type and NahG plants (Nawrath and Métrax, 1999). Here, we describe the isolation of the EDS5 gene by positional cloning. The predicted protein is a membrane protein that shows homology with MATE (multidrug and toxin extrusion) transporters.

RESULTS

Identification and Characterization of the EDS5 Gene

EDS5 was mapped ~2 centimorgan (cM) from the simple sequence length polymorphism (SSLP) marker nga1107 (Nawrath and Métrax, 1999). Analysis of 48 eds5 plants selected from a Landsberg erecta (Ler) × eds5 F2 population identified the SSLP markers F20D10-45.5 and F23K16-28.4 as the closest markers flanking the eds5 mutation on each side. Screening of 1060 randomly chosen plants from a Ler × eds5-3 F2 mapping population with the SSLP markers F20D10-45.8 and F23K16-28.4 identified 39 plants having a recombination in the interval. By using these 39 recombinant plants, EDS5 was found to be located 0.38 cM from nga1107 and 0.2 cM from CCR1. By using additional markers, EDS5 was positioned at an equal distance of 0.09 cM between two SSLP markers located at positions 49.5 and 79.3 kb of the bacterial artificial chromosome (BAC) F19H22, defining a 30-kb interval. Four of six genes present on the annotated sequence of the 30-kb region of BAC F19H22 were examined by RNA gel blot analysis. Gene 130 (At4 g39030) was identified as the putative EDS5, because this gene was pathogen inducible and showed a lower transcription level in all eds5 mutant alleles compared with wild-type plants, as shown below. An overview of the mapping strategy is given in Figures 1A and 1B.

To identify the structure of the EDS5 gene, a 1.4-kb fragment was amplified by reverse transcrip-tase–mediated polymerase chain reaction (RT-PCR) according to the annotated sequence of gene F19H22.130 (At4 g39030). Direct sequencing of the RT-PCR product identified an additional intron at the 3′ end of the gene, indicating that the end of the coding region had not yet been identified. Four additional introns were identified using an internal forward primer close to the predicted end and several reverse primers designed from the genomic sequence. The stop codon and 250 bp of the 3′ untranslated region of EDS5 were characterized with a reverse primer positioned 780 bp downstream of the annotated stop codon of the F19H22.130 gene. The 5′ untranslated region was characterized by RT-PCR using primers at positions −100, −150, −220, and −320 bp from the putative ATG start site. Products of the expected lengths were obtained in all reactions, except

![Figure 1. Positional Cloning and Structure of EDS5.](image)
EDS5 Encodes a MATE Transporter

BLAST analyses with the full-length EDS5 sequence indicate a significant homology of EDS5 with DINF (DNA damage-inducible gene F) of *Escherichia coli*. DinF is induced by treatments with DNA damage-inducing agents, such as UV-C light (Kenyon and Walker, 1980). However, the DINF function has not yet been identified (Walker, 1995; G.C. Walker, personal communication). Recently, DINF was shown to be homologous with NorM of *Vibrio parahaemolyticus* (Brown et al., 1999).
NorM is the only biochemically characterized member of the MATE protein family, and it has been found to pump antimicrobial agents out of bacterial cells in exchange for sodium (Morita et al., 1998, 2000). A second MATE protein to which a possible transporter function could be assigned is the ethionine resistance protein ERC1 from Saccharomyces cerevisiae (Shiomi et al., 1991; Brown et al., 1999). So far, 56 MATE proteins have been identified in the Arabidopsis genome and are presented in the Arabidopsis membrane protein library (www.cbs.umn.edu./Arabidopsis).

Dual sequence alignments with the T-coffee program among EDS5, ERC1, NORM, and DINF showed that all of the proteins share a limited sequence homology, having between 15 and 25% identical amino acids and between 50 and 60% homologous amino acids in the protein part covering the membrane-spanning domains of the protein. Both NORM and DINF are predicted to have 12 transmembrane domains (TMDs), whereas EDS5 and ERC1 have an additional hydrophilic domain at the N terminus. Interestingly, although these N-terminal domains share no explicit sequence homology, both contain stretches rich in glutamic acid (ERC1, 10 Glu in 18 amino acids; EDS5, 9 Glu in 12 amino acids).

Further sequence comparisons have been restricted to the region homologous with the TMDs of NORM/DINF. A comparison of EDS5 with DINF and NORM is shown in Figure 4. The homology of EDS5 with DINF was the highest (19% identical, 55% homologous), followed by NORM (18% identical, 53% homologous). The ERC1 of yeast shows a lower homology with EDS5 than with the bacterial proteins (16% identical, 51% homologous), although ERC1 also shows close homology with NORM (22% identity, 69% homologous) and DINF (22% identical, 55% homologous), indicating that ERC1 and EDS5 might have diverged independently from DINF and NORM.

The protein structure of EDS5 is predicted to include a coil domain at the hydrophilic N terminus, whereas the rest of the protein forms nine to 11 membrane-spanning domains (see Methods for the programs used). Program PRED-TMR identified 11 membrane-spanning domains correlating to TMDs 1 to 7 and 9 to 12 in the NORM protein (Pasquier et al., 1999). TMD 8 of NORM was not identified in EDS5 by any program, despite good sequence homology in this region (Figure 4).

**EDS5 Expression and Accumulation of SA after Exposure to UV-C Light**

SA has been reported to accumulate after exposure of tobacco to UV-C light (Yalpani et al., 1994). UV-C light also is a good inducer of SA biosynthesis in Arabidopsis. Figure 5A shows the accumulation of free SA in Arabidopsis leaves after 20 min of UV-C light exposure. At the start of the experiment, in plants having a low amount of free SA, the expression level of EDS5 also was very low, but detectable, after prolonged exposure to x-ray film (Figures 5A and 5B). The accumulation of free SA started ~4 hr after the beginning of the UV-C light treatment, increased during the next 8 hr, and stabilized for at least 2 days. Conjugated SA increased in parallel but continued to accumulate even when the amount of free SA did not increase further (data not shown). The EDS5 transcript increased 2 hr before the first increase in SA was observed, indicating that increased EDS5 expression might be part of the functional mechanism for the induction of SA biosynthesis. In contrast, PR-1 expression started 12 to 24 hr after UV-C light exposure once SA had accumulated to higher levels (Figure 5B).

**Expression of EDS5 after Treatment with SA**

The expression of several defense genes, such as PAD4 or EDS1 (Falk et al., 1999; Jirage et al., 1999), has been shown to be regulated by SA. Therefore, we tested the effect of SA on EDS5 expression. Applied either as a soil drench or a leaf
spray, SA enhanced **EDS5** expression between 6 and 12 hr after treatment followed by a decrease in transcript accumulation (Figure 5C). Thus, as in **PAD4** and **EDS1**, the transcriptional regulation of **EDS5** might involve a positive feedback regulation loop by SA.

**Expression of EDS5 after Inoculation with *P. syringae***

Inoculation with the avirulent strain of *P. syringae* carrying the *avrRpt2* gene led to an increase in the expression of **EDS5** starting 2 hr after pathogen inoculation, reaching a maximum at 8 hr and decreasing slowly during the next 40 hr (Figure 6A). **EDS5** was induced earlier than **PR-1**, whose transcription level began to increase only 6 hr after pathogen inoculation and was maximal between 24 and 32 hr (Figure 6A).

After inoculation with the isogenic virulent strain of *P. syringae*, transient expression of **EDS5** could be observed starting ~8 hr after inoculation, with a maximum between 12 and 24 hr and decreasing during the next 24 hr (Figure 6B). **EDS5** was less induced than after inoculation with the
avirulent strain. In comparison, PR-1 expression began to increase only 12 hr after pathogen inoculation and was maximal at ~32 hr (Figure 6B).

In all three eds5 mutant alleles, the expression level of EDS5 after inoculation with both strains of P. syringae was much lower than in wild-type plants but followed similar kinetics (Figure 6C and data not shown).

Expression of EDS5 in Mutants Affected in the Disease Resistance Responses after Pathogen Inoculation or UV-C Light Exposure

The expression of EDS5 after inoculation with avirulent and virulent P. syringae strains or UV-C light treatment was further characterized in several mutants affected in their response to pathogens, as presented in Figure 7.

sid2 is a SA induction–deficient mutant with a very similar phenotype to eds5 (Nawrath and Métraux, 1999; Dewdney et al., 2000). The level of expression of EDS5 in sid2 is not significantly different from that of the wild-type plants after inoculation with pathogens (Figures 7A and 7B) or after UV-C light exposure (Figure 7C), showing that SA is not essential for the accumulation of the EDS5 transcript.

Interestingly, the expression of EDS5 is nearly absent in SA-degrading NahG plants (Delaney et al., 1994) 6 and 12 hr after inoculation with avirulent or virulent P. syringae, respectively (Figures 7A and 7B), but it is normal after UV light exposure (Figure 7C). This finding is in sharp contrast to the expression of EDS5 in inoculated sid2 plants. However, 24 and 48 hr after inoculation with P. syringae carrying the avrRpt2 gene, NahG and Columbia expressed EDS5 to approximately the same extent (data not shown). This indicates that NahG plants might display some uncharacterized differences in addition to having very low SA levels, for example, by the unspecific action of the SA hydroxylase on potential signaling compounds other than SA (Cameron, 2000). These hypothetical differences might delay EDS5 accumulation in NahG plants.

The npr1 mutant has a block in the resistance pathway downstream of SA and accumulates higher amounts of SA than wild-type plants after inoculation with P. syringae DC3000 pv tomato carrying the avrRpt2 gene and possibly upregulating SA biosynthesis in a feedback loop (Shah et al., 1997). The npr1 mutation did not influence the expression level of EDS5 significantly after inoculation with this pathogen (Figures 7A and 7B) or UV-C light exposure (Figure 7C). Thus, NPR1 does not affect the control of EDS5 expression.

The pad4 mutant displays low amounts of SA and camalexin as well as enhanced disease susceptibility to the virulent strain of P. syringae DC3000 pv tomato (Glazebrook et al., 1997). EDS5 expression was found to be very low after inoculation with virulent P. syringae (Figure 7A) and reduced after inoculation with avirulent P. syringae (Figure 7B) or exposure to UV-C light (Figure 7C), indicating that PAD4 is in-

Figure 5. SA Accumulation and EDS5 Expression after Treatment with UV-C Light and EDS5 Expression after Exogenous Application of SA.

(A) and (B) Accumulation of free SA (A) and expression of EDS5 and PR-1 (B) in Col plants were measured in parallel after UV-C light treatment at the indicated times. In (B), a long-term exposure to x-ray film is indicated by an asterisk. FW, fresh weight.

(C) Col plants were treated by drenching the soil with 0.2 mM Na-ASA, and the plants were harvested after the times indicated. Values represent means of three to five measurements ±SE from one representative experiment.

In (B) and (C), PR-1 expression was studied in parallel to EDS5 expression. The 18S rDNA probe was used to evaluate uniform loading. The experiments were repeated twice with similar results. For (A), the absolute amounts of the SA accumulated varied somewhat from experiment to experiment, although the general shape of the curve was identical.
In (Century et al., 1995; A. Shapiro, personal communication). The transcription of EDS5 after pathogen inoculation or exposure to UV-C light involves in the transcriptional regulation of EDS5 after either pathogen inoculation or exposure to UV-C light, indicating that NDR1 also is involved in the SA-dependent pathogen response pathway as well as in the ethylene and jasmonate response pathways.

**DISCUSSION**

To characterize the biosynthesis of SA, we have used a mutational approach whereby Arabidopsis plants infected with an avirulent *P. syringae* were screened for their inability to accumulate SA. This led to the discovery of two mutants, *sid1* (allelic to *eds5*; Rogers and Ausubel, 1997) and *sid2*, both of which are characterized by low levels of SA, low PR-1 expression, and enhanced disease susceptibility after infection (Nawrath and Métraux, 1999). Therefore, EDS5 encodes a protein with an essential function in the SA-dependent pathogen response pathway as well as in the ethylene and jasmonate pathways.

**Figure 6. EDS5 Expression after Pathogen Inoculation.**

EDS5 and PR-1 transcript accumulation in wild-type plants at different times after inoculation with an avirulent strain of *P. syringae* carrying the *avrRpt2* gene (A) and after inoculation with the isogenic virulent *P. syringae* (B). In (A), a short exposure time is shown at right to clarify overexposed parts of the film to the left. (C) shows EDS5 transcript accumulation in wild-type and *eds5* mutant alleles 8 hr after inoculation with *P. syringae* carrying the *avrRpt2* gene. The 18S rDNA probe was used to evaluate uniform loading. The experiments were repeated once with similar results. M, mock inoculation; I, pathogen inoculation.
carrying the of nearly maximal expression: 8 hr after inoculation with the isogenic virulent strain [C] sure to UV-C light (Mutants in the Col background were inoculated with the isogenic virulent strain [D]. Lieberherr and J.-P. Métraux, unpublished data). These kinetics suggest that the accumulation of EDS5 transcript might be involved functionally in the accumulation of SA and plant defense after both UV-C light exposure and pathogen induction. The expression data are summarized in the following model. After either UV-C light exposure or pathogen inoculation, the increase in the transcript accumulation in a timely, coordinated manner (Figure 8). The overall speed of these events, however, depends strongly on the type of signal. Whether the same hierarchy of regulation is found at the protein level will have to be investigated in the future.

EDS5 expression also was induced by relatively high concentrations of exogenous SA. The effect of SA was slower than that of UV-C light (onset of expression 6 and 2 hr after treatment, respectively). Interestingly, the transcript accumulation of EDS5 occurred only transiently during the first hours after induction by UV-C light and pathogens and returned close to basal levels after 48 hr, despite high endogenous concentrations of SA (Figures 5 and 6). In addition, EDS5 transcript accumulation was normal in the sid2 mutant, which accumulated low amounts of SA after induction. Therefore, the biological relevance of the SA-mediated expression of EDS5 is not obvious. At optimal concentrations, it might regulate the extent of transient EDS5 expression by a positive feedback loop.

EDS5 encodes a protein of 543 amino acids with a predicted structure that includes nine to 11 membrane-spanning domains and a coiled coil at the N terminus. Its protein structure and its sequence homology with MATE proteins provide evidence that EDS5 might be a transporter. Of the 56 proteins that have been classified as MATE proteins in the Arabidopsis Membrane Protein Library database, none had a biological function assigned until very recently. The mutant transparent testa 12 blocked in flavonoid biosynthesis has been shown to be defective in a MATE protein potentially acting as a vacuolar flavonoid transporter (Debeaujon et al., 2001). Furthermore, AFL5 is a recently identified MATE transporter that renders Arabidopsis resistant to toxins (Diener et al., 2001). Thus, MATE proteins are involved in the transport of different kinds of organic molecules in plants.

The nature of the substances transported by EDS5 remains unknown. NORM, the only biochemically characterized member of the MATE proteins, is a Na+–driven antimicrobial efflux pump (Morita et al., 2000). It is possible that EDS5 transports organic molecules against Na+. These organic molecules could be part of the signal transduction cascade, possibly products of EDS1 and PAD4. It also may be possible that EDS5 has evolved to transport phenolic compounds that are precursors for the biosynthesis of SA.

Interestingly, only six of the 56 MATE proteins of Arabidopsis have an extended hydrophilic region of ~100 amino acids at the N-terminal end. The MATE proteins of yeast also have this feature. It is possible that the transport activity is regulated by factors binding to this hydrophilic domain, possibly forming a coiled coil in EDS5.

Among the different classes of MATE proteins, EDS5 is most closely related to DINF proteins. In E. coli, DINF is induced after exposure to DNA-damaging agents, including UV-C light, an interesting parallel to EDS5. DinF is part of an operon comprising genes involved in the save our souls (SOS) response (Kenyon and Walker, 1980), a mechanism leading to DNA damage tolerance in prokaryotes (Little and

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**Figure 7.** EDS5 Transcript Accumulation in Different Arabidopsis Mutants.

Mutants in the Col background were inoculated with *P. syringae* DC3000 pv tomato carrying the avrRpt2 gene (A), with the isogenic virulent strain (B), or exposed to UV-C light (C) and compared with treated Col plants. In (D), the eds1 mutant in the Ler background was inoculated with the isogenic *P. syringae* strain carrying the avrRps4 gene (left) and was treated with UV-C light (right) and compared with treated Ler plants. The plants were harvested at the time of nearly maximal expression: 8 hr after inoculation with *P. syringae* carrying the avrRpt2 gene (A) or avrRps4 (D), 24 hr after inoculation with the isogenic virulent *P. syringae* strain (B), and 7 hr after exposure to UV-C light (C) and (D). The 18S rDNA probe was used to evaluate uniform loading. M, mock inoculation; I, pathogen inoculation; C, control; T, UV-C light treatment.
UV-C light

(PAD4)

(NDR1)

Figure 8. Model for the Transcriptional Regulation of EDS5.

Treatment with UV-C light or inoculation with different strains of P. syringae induce the transcript accumulation of EDS5 when EDS1, PAD4, and NDR1 are functional. An increase in EDS5 expression is followed by the accumulation of SA and PR-1 expression. The gene names shown in parenthesis have different strengths of impact on EDS5 transcription depending on the inducer; their order of action has not been determined.

EDS5 Encodes a MATE Transporter

Mount, 1982; Walker, 1995). The genes belonging to this operon are involved in recombination and DNA repair (Walker, 1995). DINF has been found in Gram-negative bacteria, Gram-positive bacteria, and archaeabacteria (Bouyoub et al., 1995). Such conservation during evolution suggests that DINF proteins fulfill an important physiological role. In Streptococcus pneumoniae, DINF belongs to an operon induced by competence, also a process requiring DNA recombination events. It is hypothesized that DINF might function in increasing genetic exchanges that allow a better adaptation to environmental conditions (Mortier-Barrière et al., 1998), but the potential transport function of DINF has not been characterized further and it is not known if DINF is involved directly in processes requiring DNA recombination. Because eds5 mutants have not been found to be more sensitive to UV-C light irradiation (data not shown), it remains to be seen if EDS5 is involved in decreasing DNA damage in plants.

Plants have evolved to use functions for building the defense network against pathogens that have parallels in all kingdoms of organisms: R genes and the transcriptional activation of SA and PR-1 expression. The gene names shown in parenthesis have different strengths of impact on EDS5 transcription depending on the inducer; their order of action has not been determined.

METHODS

Plants and Growth Conditions of Plants and Bacteria

Arabidopsis thaliana plants, accessions Columbia (Col) and Landsberg erecta (Ler), were used in these experiments. The isolation of the mutant alleles eds5-1 and eds5-2 was as described by Rogers and Ausubel (1997) and Volko et al. (1998), respectively. The isolation of eds5-3 (sid1) and the allelism tests to eds5-1 and eds5-2 were reported by Nawrath and Métraux (1999). sid2 was described by Nawrath and Métraux (1999). Other mutants/plants were obtained from the following persons/institutions: pad4-1, ndr1-1, and npr1-1 from J. Glazebrook (Torrey Mesa Research Institute, San Diego, CA), A. Shapiro (University of Delaware, Newark), and X. Dong (Duke University, Durham, NC), respectively; etr-1 and jar-1 from the Arabidopsis Biological Resources Center (Columbus, OH); NahG plants from J. Ryals (Paradigm Genetics, Research Triangle Park, NC); and eds1-2 and Ler from Jane Parker (Sainsbury Laboratory, John Innes Centre, Norwich, UK). All mutants mentioned above are in the Col background, except edd1, which is in the Ler background. Pseudomonas syringae DC3000 pv tomato and the isogenic strains carrying the avrRpt2 or avrRps4 gene were used for pathogen inoculations. Plants and P. syringae were grown as described by Nawrath and Métraux (1999). Agrobacterium tumefaciens strain GV3101 MP90 was used for the transformation of Arabidopsis.

Isolation of RNA and DNA

For RNA gel blot analysis, RNA was isolated as described previously (Nawrath and Métraux, 1999). For reverse transcriptase-mediated polymerase chain reaction (RT-PCR), RNA was isolated from plants 24 hr after inoculation with P. syringae as described by Nawrath and Métraux (1999), except that the RNA was treated with DNase in the presence of the RNase inhibitor rRNAsin (Promega) for 1 hr at 37°C and purified by phenol/chloroform followed by ethanol precipitation. Genomic DNA used for mapping was prepared by fast isolation methods for PCR (Edwards et al., 1991; Klimyuk et al., 1993).

Inoculation with Bacteria and Treatments with UV Light and Salicylic Acid

For RNA gel blot analysis, 4- to 5-week-old plants were syringe inoculated with a suspension of 2 × 10^9/mL P. syringae DC3000 pv tomato or of the isogenic strains carrying the avrRpt2 or avrRps4 gene. Two- to 3-week-old plants were exposed to UV-C light (254 nm) at 30 cm for 20 min in the dark and then placed in continuous light until harvest. Two- to 3-week-old plants were treated with salicylic acid (SA) either by adding Na-SA to the soil at a final concentration of 0.2 mM or by spraying a 0.01% Silwet L-77 solution (OSI Specialties, Inc., Meyrin, Switzerland) containing Na-SA at 1.0 or 3.3 mM on the shoots. As controls for the second type of treatment, plants were sprayed with 0.01% Silwet L-77 alone.

Mapping, Isolation, and Identification of the EDS5 Gene

EDS5 was mapped to the lower arm of chromosome 4 near the simple sequence length polymorphism (SSLP) marker nga1107 (Nawrath and Métraux, 1999). Genetic fine mapping of the EDS5 gene was performed using additional SSLP and cleaved amplified polymorphic sequence markers (published at http://www.arabidopsis.org). Additional markers were generated by analysis of sequenced bacterial artificial chromosomes (BACs) for simple sequence islands and tested for SSLPs between the Arabidopsis accessions Col and Ler. A total of 1060 randomly selected F2 plants from the cross Ler ×
eds5-3 were screened with the flanking markers F20D10-45.8 and F23K16-28.4. The marker F20D10-45.8 was amplified using the oligonucleotides 5’-GTTGTCCCCATGCGAAAG-3’ and 5’-TCACTAGCTGAGAAGA-3’, giving fragments of 186- and 175-bp fragments in Col and Ler, respectively, whereas the marker F23K16-28.4 was amplified using oligonucleotides 5’-CGCAATTTTACATGTTATCTCAT-3’ and 5’-AGGTTATCTATGCTGGATTTA-3’, resulting in 205- and 220-bp fragments in Col and Ler, respectively. The genotype of the F2 plants having a recombination in this interval was determined in the F3 population after selfing by measurements of total SA 3 days after inoculation with P. syringae carrying the avrRpt2 gene. The EDS5 gene was determined to be flanked by the SSLP markers at position 49.4 and 79.3 kb of BAC F19H22. The marker F19H22-49.4 was amplified using the oligonucleotides 5’-TCCTATTAGGAAATGTTG-3’ and 5’-CAGCAGGGTATTTA-3’, giving fragments of 226 bp (Col) and 216 bp (Ler), whereas the marker F19H22-79.3 was amplified using the oligonucleotides 5’-ATACATGTCAGGCTGCTAT-3’ and 5’-AAAATACACGACTAGGTTTC-3’, giving fragments of 236 bp (Col) and 240 bp (Ler).

A genomic sequence was amplified from BAC F19H22 with the ExpanD High Fidelity PCR Amplification System (Boehringer, Mannheim, Germany) using the oligonucleotides 5’-GGAACTTCTTCAAT-3’ and 5’-GGAACTACAGGTGTCGAAGAG-3’, located 970 and 830 bp upstream from the ATG, respectively, and the oligonucleotide 5’-CGAATTTTACATGTTATCTCAT-3’, located 200 bp downstream of the stop codon. These primers introduced EcoRI sites that were used to clone the exons of the EDS5 gene. The sequence data were sequenced directly as RT-PCR products. The sequence data were determined in the F3 population after selfing by measurements of total SA 3 days after inoculation with P. syringae carrying the avrRpt2 gene. The EDS5 gene was determined to be flanked by the SSLP markers at position 49.4 and 79.3 kb of BAC F19H22. The marker F19H22-49.4 was amplified using the oligonucleotides 5’-TCCTATTAGGAAATGTTG-3’ and 5’-CAGCAGGGTATTTA-3’, giving fragments of 226 bp (Col) and 216 bp (Ler), whereas the marker F19H22-79.3 was amplified using the oligonucleotides 5’-ATACATGTCAGGCTGCTAT-3’ and 5’-AAAATACACGACTAGGTTTC-3’, giving fragments of 236 bp (Col) and 240 bp (Ler).

### Characterization of the EDS5 cDNA

RT-PCR was performed with the Access RT-PCR system from Promega. The 5’ end of the cDNA was determined by RT-PCR using primers located 100, 150, 220, and 320 bp upstream from the predicted ATG on the annotated genomic sequence. The 3’ end of the cDNA was determined by RT-PCR using primers located 140, 380, 660, and 780 bp downstream of the stop codon of the annotated genomic sequence. The cDNAs of wild-type and mutant alleles were sequenced directly as RT-PCR products. The sequence data were determined in the F3 population after selfing by measurements of total SA 3 days after inoculation with P. syringae carrying the avrRpt2 gene. The EDS5 gene was determined to be flanked by the SSLP markers at position 49.4 and 79.3 kb of BAC F19H22. The marker F19H22-49.4 was amplified using the oligonucleotides 5’-TCCTATTAGGAAATGTTG-3’ and 5’-CAGCAGGGTATTTA-3’, giving fragments of 226 bp (Col) and 216 bp (Ler), whereas the marker F19H22-79.3 was amplified using the oligonucleotides 5’-ATACATGTCAGGCTGCTAT-3’ and 5’-AAAATACACGACTAGGTTTC-3’, giving fragments of 236 bp (Col) and 240 bp (Ler).

A genomic sequence was amplified from BAC F19H22 with the ExpanD High Fidelity PCR Amplification System (Boehringer, Mannheim, Germany) using the oligonucleotides 5’-GGAACTTCTTCAAT-3’ and 5’-GGAACTACAGGTGTCGAAGAG-3’, located 970 and 830 bp upstream from the ATG, respectively, and the oligonucleotide 5’-CGAATTTTACATGTTATCTCAT-3’, located 200 bp downstream of the stop codon. These primers introduced EcoRI sites that were used to clone the exons of the EDS5 gene. The sequence data were sequenced directly as RT-PCR products. The sequence data were determined in the F3 population after selfing by measurements of total SA 3 days after inoculation with P. syringae carrying the avrRpt2 gene. The EDS5 gene was determined to be flanked by the SSLP markers at position 49.4 and 79.3 kb of BAC F19H22. The marker F19H22-49.4 was amplified using the oligonucleotides 5’-TCCTATTAGGAAATGTTG-3’ and 5’-CAGCAGGGTATTTA-3’, giving fragments of 226 bp (Col) and 216 bp (Ler), whereas the marker F19H22-79.3 was amplified using the oligonucleotides 5’-ATACATGTCAGGCTGCTAT-3’ and 5’-AAAATACACGACTAGGTTTC-3’, giving fragments of 236 bp (Col) and 240 bp (Ler).

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