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NDR1, a Pathogen-Induced Component Required for *Arabidopsis* Disease Resistance

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Plant disease resistance (*R*) genes confer an ability to resist infection by pathogens expressing specific corresponding avirulence genes. In *Arabidopsis thaliana*, resistance to both bacterial and fungal pathogens, mediated by several *R* gene products, requires the *NDR1* gene. Positional cloning was used to isolate *NDR1*, which encodes a 660–base pair open reading frame. The predicted 219–amino acid sequence suggests that NDR1 may be associated with a membrane. *NDR1* expression is induced in response to pathogen challenge and may function to integrate various pathogen recognition signals.

Genetic analyses of disease resistance in plants show that resistance to pathogens is often highly specific, requiring single corresponding genetic loci in both the plant and the pathogen (*1*). Disease resistance genes cloned from diverse plant species such as tomato, rice, and *Arabidopsis thaliana* encode proteins that share one or more similar motifs (*2*). These motifs include leucine-rich repeat regions (implicated in protein-protein interactions) (*3*), nucleotide-binding sites, and kinase domains, all of which predict a role for resistance genes as components in signal transduction pathways. These genes confer resistance to a variety of pathogens, including bacteria, fungi, viruses, and nematodes, which suggests a conserved mechanism of plant disease resistance. Therefore, it is possible that the signal transduction pathways used by the different resistance gene products converge at some point. We report the cloning of *NDR1* from *Arabidopsis*, a gene that functions in common among several resistance responses.

The *NDR1* locus is required for resistance to both the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*) and the fungal pathogen *Peronospora parasitica* (*4*). Mutation of *NDR1* causes susceptibility to numerous strains of these pathogens. Thus, NDR1 represents a strong candidate for a conserved signal transduction element required for avirulence (avr) gene–specific disease resistance. *NDR1* is located on *Arabidopsis* chromosome three, in an ~8.5-centimorgan (cM) interval between restriction fragment length polymorphism (RFLP) markers g6220 and g4711 (*4*). Fine-structure mapping with RFLP and polymerase chain reaction (PCR)–based markers further delimited the genomic region carrying *NDR1* (Fig. 1A) (*5*). An overlapping set of yeast artificial chromosome (YAC) clones spanning ~1200 kb was constructed (Fig. 1B) (*6*). Two YAC clones, CIC3D12 and CIC7E1, together spanned *NDR1*, as determined by recombination analysis. A plant-transformation competent cosmid library from each of these two YAC clones was generated, and a cosmid contig was constructed (Fig. 1C) (*7*). An approximate 1-kb deletion was uncovered in the *ndr1-1* fast-neutron–generated mutant (*4*) when cosmid FH6 from the CIC3D12 library was

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Table 1. Asexual sporulation (measured as the mean number of sporangiophores per cotyledon; maximum of 20 sporangiophores counted per cotyledon) by three incompatible isolates of *Peronospora parasitica* on Col-0, the mutant *ndr1-1*, and two transformed lines of *ndr1-1*. *RPP* resistance specificities for each isolate are indicated. The cotyledon assay used has been described previously (*31*). SEM, standard error of the mean; n, number of seedlings inoculated.

Arabidopsis line	*Peronospora* isolate								
	Cala2-*RPP2*			Emwa1-*RPP4*			Emoy2-*RPP4*		
	Mean	SEM	(n)	Mean	SEM	(n)	Mean	SEM	(n)
Col-0	0.18	0.07	(76)	4.01	0.28	(86)	5.94	0.40	(58)
ndr1-1	0.95	0.11	(85)	10.54	0.57	(95)	14.66	0.60	(86)
ndr1-1 FH6	0.03	0.03	(87)	4.89	0.29	(128)	7.00	0.41	(93)
ndr1-1 CB17	0.69	0.10	(100)	10.91	0.53	(100)	14.03	0.62	(87)

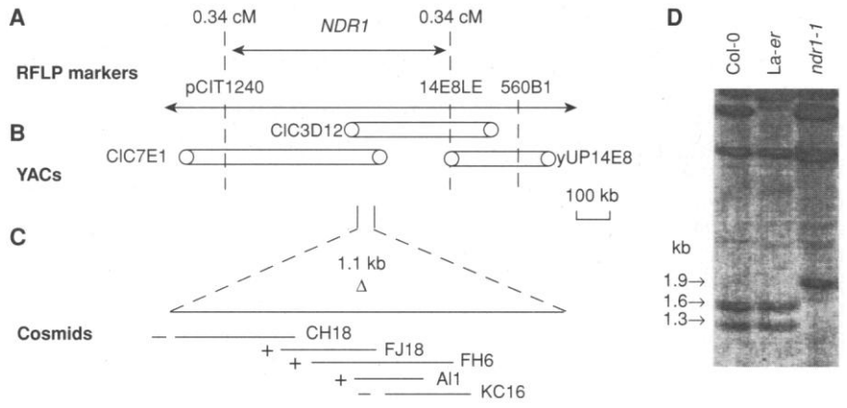

Fig. 1. Genetic mapping and positional cloning of *NDR1*. (**A**) Fine-structure RFLP map. Recombinant analysis delimited the genomic region containing *NDR1* to a section flanked by RFLP marker pCIT1240 and ARMS (*Arabidopsis* RFLP mapping set) marker 560B1 (*5*). (**B**) YAC contig. Genetic mapping of the insert ends from the YAC clones demonstrated that the contig spanned the *NDR1* locus (*6*). An RFLP marker (14E8LE) derived from one end of yUP14E8 further narrowed the physical genomic region containing *NDR1* to a 0.68-cM interval. (**C**) Cosmid contig. Cosmids derived from CIC3D12 were organized into an overlapping set that spanned *NDR1*. The *ndr1-1* mutant was genetically transformed with these cosmids and tested for complementation by HR analysis (+, HR restored; –, no HR) (*7*). (**D**) DNA gel blot demonstrates an ~1-kb deletion in the mutant *ndr1-1*. A 14-kb Eco RI *Arabidopsis* DNA fragment from cosmid FH6 was radiolabeled and used as a hybridization probe against Hind III–digested Col-0, La-*er*, or *ndr1-1* genomic DNA. The *ndr1-1* lane shows the deletion of an ~1-kb fragment containing a Hind III site resulting in the larger 1.9-kb single fragment.

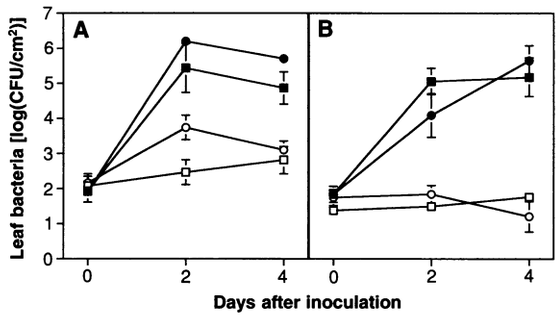

Fig. 2. Growth of avirulent *Pst* strain DC3000 within cosmid-complemented *ndr1-1 Arabidopsis*. (**A**) *Pst* DC3000 (*avrRpt2*). (**B**) *Pst* DC3000 (*avrRpm1*). ●, *ndr1-1* mutant; ■, *ndr1-1* transformed with noncomplementing CB17 cosmid; ○, wild-type Col-0; and □, *ndr1-1* transformed with complementing FH6 cosmid. T_3 homozygous plant lines were derived from selfing progeny of a single Col-0 *ndr1-1/ndr1-1* transformant heterozygous for kanamycin resistance. Plants were inoculated by vacuum infiltration, and bacterial growth in leaves was monitored as described (*27*). CB17 cosmid does not contain NDR1 ORF (Fig. 1C). Sample means and standard deviations are shown from a representative experiment.

```
1    M N N Q N E D T E G G R N C C T C C L S F I F T A G L T S L F L W L S L R
38   A D K P K C S I Q N F F I P A L G K D P N S R D N T T L N F M V R C D N P
75   N K D K G I Y Y D D V H L N F S T I N T T K I N S S A L V L V G N Y T V P
112  K F Y Q G H K K K A K K W G Q V K P L N N Q T V L R A V L P N G S A V F R
149  L D L K T Q V R F K I V F W K T K R Y G V E V G A D V E V N G D G V K A Q
186  K K G I K M K K S D S S F P L R S S F P I S V L M N L L V F F A I R
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Fig. 3. Primary structure of NDR1-predicted protein (*28*). Putative transmembrane domains are underlined. The entire NH_2-terminal portion of the protein through amino acid Asn^{179} (indicated by the arrow) is deleted in *ndr1-1*, as well as a portion of the upstream DNA (GenBank accession number AF021346). Molecular alterations in *ndr1-2* and *ndr1-3* alleles convert Trp^{124} and Phe^{31}, respectively, to premature stop codons and are indicated by asterisks. See the GenBank entry (www.mcbi.nlm.nih.gov/Entrer/nucleotide.html) for nucleotide sequence and base pairs altered in the mutant alleles.

used as a ^{32}P-labeled probe (Fig. 1D). To determine if *NDR1* was contained in the deleted region, *ndr1-1* plants were transformed with cosmids spanning the deletion and tested for complementation with a hypersensitive response (HR) assay (*7*). Wild-type Col-0 plants react with an HR to *P. syringae* pv. *maculicola* (*Psm*) expressing *avrRpt2*, whereas *ndr1-1* mutant plants do not. Cosmids spanning the deleted region (Fig. 1C) restored wild-type HR to *Psm* (*avrRpt2*) in *ndr1-1* plants. In planta bacterial growth analyses (Fig. 2) and cotyledon sporulation assays with *P. parasitica* (Table 1) demonstrated restored, heritable resistance in the complemented transformed plants.

Sequencing of wild-type Col-0 genomic DNA revealed a single 660-base pair open reading frame (ORF) in the region spanned by the deletion in the *ndr1-1* mutant (*8*). Sequencing of the additional mutant alleles *ndr1-2* and *ndr1-3* (*9*) also revealed alterations in this ORF (Fig. 3). The ORF predicts a 219–amino acid gene product (Fig. 3), which shows identity to one *Arabidopsis* expressed sequence tag (GenBank accession number T21313). This cDNA clone was obtained from the *Arabidopsis* Biological Resource Center and was used to probe RNA gel blots (Fig. 4). The size of the hybridizing RNA from wild-type Col-0 plants is in agreement with the size of the ORF, indicating that the full-length gene is contained in the single ORF without introns. The message is absent in *ndr1-1* mutant plants (*10*). Accumulation of *NDR1* mRNA is up-regulated by both virulent and avirulent bacteria relative to the $MgCl_2$ control treatment in wild-type Col-0 plants (Fig. 4A) and in mutants *ndr1-2* and *ndr1-3* (*10*). Over a period of 48 hours, up-regulation of *NDR1* was seen as early as 4 hours after inoculation, with maximal expression at 8 hours (Fig. 4B). Therefore, *NDR1* is a classically induced defense response gene which is genetically required for resistance.

SBASE library (*11*) analysis of the NDR1-predicted amino acid sequence identified two putative transmembrane domains similar (up to 85%) to membrane-spanning regions in proteins such as the 6K protein of Ockelbo virus (a Sindbis virus) and the inositol 1,4,5-trisphosphate receptor protein from various species. In NDR1, the putative transmembrane domains span amino acids 19 through 36 and 202 through 218 (Fig. 3). These similarities suggest that NDR1 may be a membrane-associated protein; however, the subcellular location of NDR1 is not known.

BLAST searches (*12*) revealed limited similarity with two tobacco genes, *hin1* (*13*) and clone NG2 (*14*), which are cor-

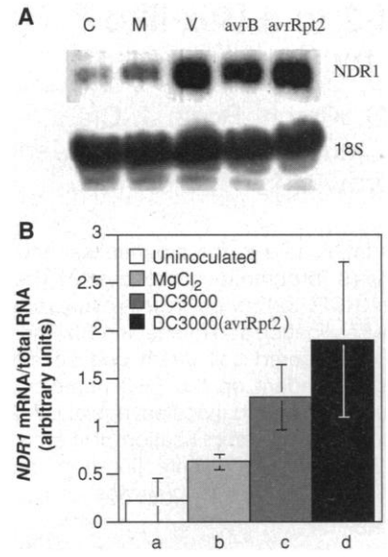

Fig. 4. (**A**) RNA gel blot showing expression of *NDR1* message in WT Col-0 *Arabidopsis* plants. Five-week-old plants grown under short-day conditions in a growth chamber were vacuum infiltrated with either a 10 mM $MgCl_2$ control or 1×10^7 CFU/ml of *Pst* DC3000, *Pst* DC3000 (*avrB*), or *Pst* DC3000 (*avrRpt2*). Plants were frozen in liquid nitrogen after an 8-hour induction period, and total RNA was extracted (Tri-Reagent, Sigma). Lanes: C, Col-0 uninduced; M, $MgCl_2$ induction control; V, virulent *Pst* DC3000 induction; avrB, avirulent *Pst* DC3000 (*avrB*) induction; *avrRpt2*, avirulent *Pst* DC3000 (*avrRpt2*). Gel blot analysis was done according to standard protocol (*29*) using Hybond-NX transfer membrane (Amersham) according to manufacturer's directions. The blot was stripped and reprobed with pea 18S ribosomal DNA (*30*) as a control for loading. The blot shown is representative of three experiments. (**B**) *NDR1* mRNA accumulation after 8 hours in uninoculated tissue (a), leaves infiltrated with $MgCl_2$ control (b), virulent DC3000 (c), or avirulent DC3000 (*avrRpt2*) (d). Data was generated by combining the results from three separate RNA gel blots and was standardized for loading by comparing with control probes for total RNA. A similar trend of RNA accumulation was seen in plants inoculated with DC3000 (*avrB*) in two separate experiments (*10*).

related with the resistance response (*15*). Both *hin1* (*13*) and NDR1 are induced by avirulent pathogens (Fig. 4). The significance of the similarities between *ndr1* and these two tobacco genes remains to be determined.

NDR1 is required for resistance to the bacterial pathogen *Pst* expressing *avrB*, *avrRpt2*, *avrRpm1*, or *avrPph3*, as well as resistance to numerous isolates of the fungal pathogen *Peronospora parasitica* (*4*), but not for expression of the resistance gene *RPS2* (*16*). Therefore, we propose that *NDR1* may encode a component in the signal transduction pathway downstream of initial pathogen recognition. Mutation of *NDR1* results in loss of resistance governed by several resistance genes. Because of their specificity, it has been speculated that resistance gene products act as receptors for avirulence signals. NDR1 may interact directly with many specific receptors to transduce the elicitor signal, or it may serve as a transporter or receptor for an elicitor signal or secondary messenger.

Several genes have been identified that are required for the activity of individual resistance genes in tomato and barley (*17*). However, *NDR1* and another *Arabidopsis* gene, *EDS1* (*18*), have been shown to be necessary for plant defense mediated by numerous resistance genes. That mutation of *NDR1* causes susceptibility to both bacterial and fungal pathogens supports a central role for NDR1 in disease resistance. Further analysis of NDR1, such as identification of important domains, interacting proteins, and cellular localization, will help make significant progress toward the goal of characterizing a complete signal transduction pathway for plant disease resistance.

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5. A segregating population from a cross between Col-0 *ndr1-1*/*ndr1-1* and La-*er NDR1*/*NDR1* plants was used for genetic mapping. Molecular markers were obtained from the *Arabidopsis* Biological Resource Center (Ohio State University) and were mapped relative to *NDR1*. Flanking PCR markers nga162 (*19*) and *gl1* (*20*) (22 cM apart) were used to rapidly prescreen large numbers of susceptible F_2 plants for recombinants. F_3 families from recombinant plants were then used to identify recombination events between RFLP markers tightly linked to *NDR1*, as well as to verify the genotype of the recombinants by disease assays (*21*).
6. Four YAC libraries (*22*) of *Arabidopsis* accession Col-0 genomic DNA were screened with 560B1 and pCIT1240 as hybridization probes. Plant DNA insert ends were isolated from YAC clones by inverse PCR and plasmid rescue as described (*23*), and were converted to RFLP markers for genetic mapping. The contig was extended until an overlapping set of YAC clones was constructed that linked the clones identified with 560B1 to those that hybridized with pCIT1240.
7. For construction of cosmid libraries, yeast DNA from clones CIC3D12 and CIC7E1 was partially digested with Sau 3A, size fractionated (to 15 to 20 kb), and cloned into the Bam HI site of the binary vector pCLD04541 (*24*). Clones carrying *Arabidopsis* DNA were isolated by hybridization with RFLP markers that cosegregated with *NDR1*. Cosmids were organized into overlapping contig islands by restriction analysis and hybridization patterns. The discovery of the 1.2-kb deletion in the area spanned by cosmid FH6 focused our efforts on cosmids in that region. *Agrobacterium*-mediated transformation of *ndr1-1* plants was performed using the vacuum infiltration procedure of Bechtold *et al.* (*25*), with modifications as described by Bent *et al.* (*24*). T_1 seeds were surface-sterilized, and transformants were selected on agar-solidified Murashige-Skoog medium containing kanamycin (40 mg/ml). After 1 week, green plants were transferred to potting mix and inoculated after 3 to 5 weeks growth. The HR assay used to test for complementation is described by Kunkel *et al.* (*26*). *Psm* strain 4326 lacking an *avr* gene recognized by *Arabidopsis* plants does not cause a visible HR and was used as a negative control inoculation. Cosmids that lacked the region encompassed by the deletion (Fig. 1C) did not restore the HR resistance response in *ndr1-1* plants. Thus, the region encoding *NDR1* was delimited to a 3.5-kb section.
8. To sequence genomic DNA spanning the deletion in the *ndr1-1* mutant, we sonicated cosmid FH6 DNA, then size-selected ~1-kb fragments on agarose gels, purified them with GeneClean (Bio 101, Vista, CA), and subcloned them into the Eco RV site of pBluescript (Stratagene). Two Hind III fragments which spanned the deletion were isolated from FH6, radiolabeled, and used as probes against the subclones. DNA sequences from these subclones were obtained by the cyclic reaction termination method, using fluorescently labeled dideoxyribonucleotide triphosphates and following the instructions provided with the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Perkin-Elmer). DNA Sequencing Analysis, version 2.01 (Applied Biosystems) and SeqEd, version 1.03 (Gene Codes, Ann Arbor, MI) were used to process raw data. Sequences were aligned and analyzed with Sequencher 3.0 for the Macintosh.
9. *ndr1-2* and *ndr1-3* alleles were gifts from R. Innes.
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