MOLECULAR HOST DEFENSE: SIGNAL TRANSDUCTION

I. Elicitors and Signal transduction underlying basal defense

A. General elicitors of basal local defense responses are equivalent to PAMPs (Pathogen-Associated Molecular Patterns) that are recognized by animal innate immune systems (Nurnberger and Brunner 2002; Gomez-Gomez and Boller 2002)
   1. note the difference between general elicitors and HR-elicitors (often race-specific), e.g., Avr proteins
   2. examples of general elicitors (Hahn 1996; Felix et al. 1999; Hammond-Kosack and Jones 2000)
      a. oligogalacturonates that are ca. 12mers (Doares et al. 1995; Vidal et al. 1997)
      b. bacterial flagellin: recognized by FLS2 protein

B. FLS2 signaling pathway
   - an LRR receptor-like kinase (FLS2) in Arabidopsis recognizes a conserved N-terminal domain of eubacterial flagellins (Gomez-Gomez and Boller 2000; Asai 2002)

II. Signal transduction underlying gene-for-gene disease resistance

A. Phosphorylation cascades (Martin 2003)
   2. Downstream signaling components:
      Pt1 (Zhou 1995).
      PBS1 (Shao 2003; Swiderski 2001; Warren 1999).
      Calcium-dependent protein kinases (CDPKs) play an essential role in a plant defence response. (Romeis 2000; Romeis 2001).

B. Systemic Acquired Resistance (SAR) (Chester 1933; Ross 1961; Dean and Kuc 1985; Ward et al. 1991; Ryals et al. 1996; Hammond-Kosack and Jones 2000)
   1. Model systems include: tobacco - many pathogens, cucurbits - Colletotrichum lagenarium (Kuc et al. 1975; Dean and Kuc 1985; Madamanchi and Kuc 1991) and Arabidopsis - Pseudomonas syringae, Peronospora parasitica, etc. (Uknes et al. 1993)
b. "signal" movement and potentiation: salicylic acid (Dempsey et al. 1999)
c. evidence that salicylic acid is important in development of SAR, possibly by elevating levels of $H_2O_2$ - at least at the local level during pathogen challenge (Malamy et al 1990; Metraux et al 1990; Rasmussen et al. 1991; Gaffney et al. 1993; Delaney et al. 1994)

C. Discovery and characterization of NIM1/NPR1
1. Arabidopsis NPR1 gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. (Cao 1997; Ryals 1997).
3. NPR1 differentially interacts with members of the TGA/OBF family of transcription factors that bind an element of the PR-1 gene required for induction by salicylic acid. (Zhou 2000)
4. In vivo interaction between NPR1 and transcription factor TGA2 leads to salicylic acid-mediated gene activation in Arabidopsis. (Fan 2002).

D. Other defense signaling pathways known in Arabidopsis (Hammond-Kosack and Jones 2000; Delaney 1997; Glazebrook 1999)
1. SAR and ISR signaling
2. R proteins differ in their requirements for EDS1 and NDR1
3. SA-dependent pathway appears more important in defense against biotrophic parasites (Thomma et al. 1998)
4. JA-dependent pathway appears more important in defense against necrotrophic parasites (Thomma et al. 1998; Vidal et al. 1997)
5. Overview of defense signaling in Arabidopsis (Glazebrook 2001)

REFERENCES

*Assigned reading


Molecular host defense: Signal Transduction

I. Elicitors and signal transduction underlying basal defense
   A. General elicitors of defense responses
   B. FLS2 signaling pathway

II. Signal transduction underlying gene-for-gene disease resistance
   A. Phosphorylation cascades
   B. Systemic acquired resistance (SAR) and signaling pathways
   C. Discovery and characterization of NIM1/NPR1
   D. Other defense pathways known in Arabidopsis
A simplified model for plant signalling responses induced by various pathogen elicitors. The model illustrates the multifaceted nature of pathogen attack and the broad spectrum of elicitors produced as a consequence, ranging from non-specific elicitors (that may be loosely defined as plant PAMPs), through to highly specific elicitors (virulence effector/avirulence factors) with narrow specificity. One role of the latter may be to suppress plant mechanisms capable of responding to the former. Together with mechanisms that mask PAMPs, this may be one of the main strategies used by plant pathogens to avoid detection. Plants have evolved mechanisms to detect elicitors from both extremes of the spectrum, and whilst detection of non-specific elicitors may play a role in non-host resistance, detection of specific elicitors is required to resist pathogens that have evolved to overcome all the non-specific barriers and detection mechanisms. Nevertheless, non-host and host-specific resistance often result in the activation of similar responses and the model also illustrates the possible integration of non-host and host-specific resistance signalling pathways suggested by evidence for a number of shared signalling components. Red arrows indicate pathogen strategies for infection and black arrows indicate plant signalling for resistance. Although detection of PAMPs is shown at the cell surface, and the action of virulence effector proteins and their detection as avirulence factors is shown in the cytosol, these locations are not mutually exclusive. Abbreviations: EDS1, enhanced disease susceptibility 1; MAP, mitogen activated protein; NDR1, non-race-specific disease resistance 1; PAD4, phytoalexin deficient 4; PAMP, pathogen-associated molecular pattern; PR, pathogenesis related.
Bacterial flagellin is an elicitor recognized by FLS2

Figure 2. Alignment of N-terminal sequences of subbacterial flagellin sequences. Schematic representation of flagellin gene structure with conserved N- and C-terminal sequences and a variable middle part (top). Alignment of N-terminal sequences with consensus sequence (bottom) and the N-terminal sequence derived from the 33 kDa protein purified from Pseudomonas syringae pv tabaci.

Table 2. Alkalization response in cell cultures of different plant species

<table>
<thead>
<tr>
<th>Cell culture</th>
<th>Flagellin</th>
<th>flg22</th>
<th>flg15</th>
<th>Chitin fragmentsa</th>
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<td>Rice</td>
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<td>n.r.</td>
<td>n.r.</td>
<td>20</td>
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n.r. No response when treated with concentrations up to 1000 nM of flg22 and flg15, and up to 100 nM of flagellin, respectively.
aAlkalization in response to the chitin fragment penta-N-acetyl chitopentaose. A. thaliana cells and rice cells show much higher sensitivity for chitin fragments with a higher degree of polymerization (data not shown).
Fig. 2. Model for flagellin signalling in Arabidopsis. (a) Bacteria move on the plant surface and enter the plant via natural openings, wounds or hydathodes. A leaf is shown, but roots and stems are equally affected. (b) Bacteria multiply on the surface or in the intercellular space of the host plant. (c) Flagellin (released from bacterial flagellae) or flagellin fragments resembling flg22, are recognized directly or indirectly by the flagellin receptor at the plant plasma membrane (PM), a key part of which is FLS2. This recognition event might lead to autophosphorylation of FLS2, in analogy to the signalling modules in animals. Through unknown signalling pathways, the recognition event also induces the rapid phosphorylation of diverse downstream target proteins, presumably including calcium channels and other ion channels as well as the NADPH oxidase complex, which is involved in the release of hydrogen peroxide in conjunction with superoxide dismutase (SOD). A kinase-associated protein phosphatase (KAPP) is a negative regulator in this pathway. In addition, a MAPK cascade is activated (Fig. 1) that culminates in the activation of unknown transcription factors, causing induced expression of defence genes as well as of genes for WRKY transcription factors that might amplify the signal. In contrast to the defence mediated by resistance genes, this pathway does not lead to a hypersensitive response and cell death.
Figure 5 The flg22 MAPK cascade and specific WRKYs are important for Arabidopsis defence. 

a, Nuclear localization of WRKY29-GFP. Visualization of protoplasts expressing WRKY29-GFP (top), a red-fluorescent nuclear marker (middle), and super-imposition (bottom) without flg22. 
b, WRKY22 and WRKY29 activate early-defence genes. Protoplasts were expressing GFP, WRKY29 (29), WRKY22 (22) or WRKY42 (42). 
c, WRKY29 acts downstream of FLS2. Protoplasts were isolated from wild-type (FLS2) and mutant (fls2) leaves. 
d, Leaves expressing MEKK1, MKK4a, MKK5a or WRKY29 exhibit reduced disease symptoms after P. syringae (Ps) infection. Control was infiltrated with 10 mM MgSO4 (Mg). e, Leaves expressing MEKK1, MKK4a or WRKY29 exhibit reduced disease symptoms after B. cinerea infection on the right half of each leaf. f, The early stage of B. cinerea development was inhibited on leaves expressing MKK4a. On MKK1a-expressing leaves (MKK1a), germinated spores (s) of B. cinerea formed superficial hyphae (h) and branched appressoria (a) 2 days after infection. The fungal spores (s) formed only germ tubes (gt) on MKK4a-expressing leaves (MKK4a). Scale bars, 50 µm.
II. Signal transduction underlying gene-for-gene disease resistance

Hammond-Kosack and Jones 2000
The Arabidopsis PBS1 resistance gene encodes a member of a novel protein kinase subfamily
Michal R. Swiderski and Roger W. Innes

Figure 5. PBS1 displays autophosphorylation activity.

(a) Autophosphorylation assay. Approximately 150 ng of purified PBS1 was incubated with [32P]-ATP and then separated on a SDS polyacrylamide gel (lane 1). As a control, a sample purified in the same way, but derived from E. coli expressing the empty vector pTYB2, was assayed for kinase activity (lane 2). Lanes 3 and 4 show kinase assays containing the same pTYB2 fraction plus 10 µg myelin basic protein (lane 3) or 4 µg maltose binding protein (lane 4). Kinase activity was observed only in the PBS1-containing sample.

(b) Coomassie-stained gel. To establish the purity of the PBS1 protein preparation, and to demonstrate that protein was present in lanes 3 and 4 of the autophosphorylation assay, a parallel gel was run and stained with Coomassie Blue. Lane 1, pTYB2 vector only control. Lane 2, c. 1.5 µg of purified PBS1. Lane 3, pTYB2 vector control plus 10 µg myelin basic protein. Lane 4, pTYB2 vector control plus 4 µg maltose binding protein.
Mitogen Activated Protein Kinases - MAPKs

- Evolutionarily conserved throughout eukaryotes
- Central component of signal transduction pathways
- Specificity determined by direct protein-protein interactions
- Demonstrated role in stress and hormonal signaling in plants

**Arabidopsis**

MEKK or MAP3K → MAPKKK → MAPKK → MAPK

- MEKK or MAP3K: > 60
- M KK or MEK: 10
- MPK: 20
## Activation of MAPKs by Pto + AvrPto/AvrPtoB

### PtoR

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### PtoS

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</tbody>
</table>

### AvrPto

- Time (hr): 0 1 1
- Proteins: PtoS, PtoR, prf-3
- AvrPto

### AvrPtoB

- Time (hr): 0 1 1
- Proteins: PtoS, PtoR, prf-3
- AvrPtoB

**50 kD**
Plant MAPK cascades involved in stress and pathogen recognition

**Arabidopsis**
- flg22
- FLS2
- AtMEKK1
- AtMKK4/5
- AtMPK3/6
- response

**Tobacco**
- SA, stress, elicitors
- ?
- NtMEK2
- WIPK/SIPK
- WRKY22/29

**Tomato**
- AvrPto/AvrPtoB
- Pto
- LeMEK2 (?)
- LeWIPK/LeSIPK (?)
Two MAPK cascades, NPR1, and TGA transcription factors play a role in Pto-mediated disease resistance in tomato


Silencing of the genes shown compromises Pto-mediated resistance

RT-PCR showing decrease in transcript abundance in panel a

AvrPto / Pto / Prf

Other signaling pathways

MAPKK: ?

MAPK: MEK2 MEK1

MAP: WIPK NTF6

Transcription factors: TGA2.2 / TGA1a

Disease resistance
Virus-induced gene silencing (VIGS) identifies a MAPKKK gene required for Pto-mediated HR
Nb plants silenced for MAP3Kα show reduced HR

RT-PCR verifies reduced NbMAP3Kα transcripts
Virus-induced gene silencing of MAP3Kα suppresses the cell death associated with disease

Inoculated with Pseudomonas DC3000

Silencing of MAP3Kα reduces disease symptoms

TRV::LeMAP3Kα

TRV

TRV::LeMAP3Kα

Rio Grande-prf3 (susceptible to DC3000)
Signaling pathways leading to resistance and susceptibility share common components

Incompatible interaction

Early detection of bacteria by host, leads to plant cell death (HR).

Compatible interaction

Bacterial multiplication leads to plant cell death.

\[ \text{Pseudomonas} \]

\[ \text{Pto / AvrPto / Prf} \]

\[ \text{Other signaling pathways} \]

\[ \text{MAP3K}_\alpha \]

\[ \text{MEK2} \]

\[ \text{MEK1} \]

\[ \text{NTF6} \]

\[ \text{SIPK} \]

\[ \text{NPK1} ? \]

\[ \text{HR cell death} \]

\[ \text{Disease-associated cell death} \]
Zhang and Klessig 2001
Calcium-dependent protein kinases play an essential role in a plant defence response
Tina Romeis, Andrea A. Ludwig, Raquel Martin and Jonathan D.G. Jones

Fig. 2. Expression patterns of NtCDPK2 and NtCDPK3 after elicitation and hypo-osmotic stress. Cf-9 cell cultures and plants were treated with IF(Avr9+) or IF(Avr9-), which contains (+Avr9) or does not contain (−Avr9) the Avr9 peptide. Osmotic stress was applied by adding 2 vols of water to cells or by infiltrating water into leaves. At the time points indicated leaf samples were harvested and total RNA was isolated and used for RT–PCR as described in Materials and methods, applying 22, 24 and 22 amplification cycles with specific primers for the NtCDPK2, NtCDPK3 and WIPK gene, respectively (gene indicated on the right). Equal cDNA amounts were controlled by amplification of constitutively expressed actin gene (24 cycles).

Fig. 5. VIGS of NtCDPK2 in N.benthamiana. Seedlings were inoculated with A.tumefaciens PVX-NtCDPKCLD, containing the silencing construct, in which a 417 bp fragment of the calmodulin-like domain of the NtCDPK2 gene was integrated into the PVX genome, or an unrelated control insert (Jones et al., 1999). After 3 weeks the silencing was accomplished, and if not otherwise stated, the fourth leaf above the inoculated one was then used for further experiments. (A) To determine the degree of silencing and analyze the transcript levels, the silenced fourth leaf of PVX-treated or the equivalent leaf of an untreated plant was exposed to hypo-osmotic stress by infiltrating water. Before (−) and 1 h after the flooding stimulus (+), samples were harvested and analyzed by RT–PCR. (B) Transient expression of NtCDPK2-myc and its truncated version in CDPK-silenced plants. Different leaves of plants previously inoculated with PVX-NtCDPKCLD or leaf No. 4 of an untreated or PVX-GFP-inoculated control plant were infiltrated with A.tumefaciens NtCDPK2-myc and its truncated version in CDPK-silenced plants. Different leaves of plants previously inoculated with PVX-NtCDPKCLD or leaf No. 4 of an untreated or PVX-GFP-inoculated control plant were infiltrated with A.tumefaciens NtCDPK2-myc (Table I) as described in the legend to Figure 3 (upper panel) or with NtCDPK21–380-myc lacking the junction and calmodulin-like domain (lower panel; Harper et al., 1994). After two more days, the Agrobacterium-infiltrated area was subjected to hypo-osmotic stress by infiltrating water for 7.5 min. Leaf samples before (−) and after (+) the flooding stimulus were harvested, and extracts were prepared and analyzed by western blotting.

Fig. 6. CDPK-silenced leaves were compromised in gene-for-gene-dependent HR induction. For the onset of silencing, N.benthamiana seedlings were inoculated with A.tumefaciens PVX-NtCDPKCLD (upper row), PVX-GFP (control; middle row) or remained untreated (lower row), as described in the legend to Figure 5. After 3 weeks the fifth leaf (first column) or the fourth leaf (second to fourth column) above the primary infected one was infiltrated with A.tumefaciens 4/456/Avr4 or 9/456/Avr9, as indicated, carrying either Cf-4/Avr4 or Cf-9/Avr9 gene-for-gene combinations on one leaf half (upper half in third and fourth column) or with strain 4/456/Avr9 as control carrying Cf-4/Avr9 on the other leaf half (lower half in third and fourth column). Photos of necrosis and wilting phenotype were taken as indicated.
Systemic acquired resistance (SAR)

Dependent on salicylic acid (SA)
Requires NPR1

Independent of SA
Requires NPR1
Dependent on jasmonic acid (JA)
Dependent on ethylene (ET)

Induced Systemic Resistance (ISR)

Hammond-Kosack and Jones 2000
Induced systemic resistance

Fig. 3. Level of induced protection against infection by *Pseudomonas syringae* pv. *tomato* in different Arabidopsis genotypes. Wild-type Arabidopsis Col-0 plants, and genotypes altered in their response to either salicylic acid (NahG), jasmonic acid (jar1), ethylene (etr1) or inducers of systemic acquired resistance (SAR; npr1) were treated with non-pathogenic, induced systemic resistance (ISR)-inducing rhizobacteria by growing them in soil containing *P. fluorescens* WCS417r bacteria. SAR was induced three days before challenge inoculation by pressure infiltrating three lower leaves per plant with the avirulent pathogen *P. syringae* pv. *tomato* (avrPst) carrying the avirulence gene *avrRpt2*. Chemical treatments were performed three days before challenge inoculation by dipping the leaves of five-week-old plants in a solution containing either 100 μM methyl jasmonate (MeJA) or 1 mM of the ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC). Challenge inoculations were performed by dipping the leaves of five-week-old plants in a bacterial suspension of the virulent pathogen *P. syringae* pv. *tomato*. Four days after challenge, plants were evaluated on the severity of disease symptoms. The level of protection is given as a reduction of disease symptoms compared to control-treated plants. In contrast to pathogen-induced SAR, rhizobacteria-mediated ISR follows a salicylic acid-independent, and jasmonic acid-dependent and ethylene-dependent signalling pathway. Like SAR, rhizobacteria-mediated ISR is regulated by NPR1. Methyl jasmonate-induced protection is blocked in jar1, etr1 and npr1 plants, whereas ACC-induced protection is affected in etr1 and npr1 plants, but not in jar1 plants. This illustrates that components from the jasmonic acid response and the ethylene response act in sequence and upstream of NPR1 in activating resistance against *P. syringae* pv. *tomato*.

*Statistically significant differences in disease severity compared with the non-treated control plants (Fisher’s LSD test, α = 0.05).*

Pieterse and van Loon 1999
<table>
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<tr>
<th>Material</th>
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<th>COLTectisan Lecqvarian</th>
<th>NR</th>
<th>33</th>
<th>k</th>
<th>Part</th>
<th>Inducer organ</th>
<th>SAR</th>
<th>HR</th>
<th>System of resistance against</th>
<th>SAH genes</th>
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*NR not recorded; **SAH experiments with fab43 test were performed with these plants.*
Accumulation of SA is associated with SAR

Metraux et al. 1990

Fig. 1. Time course of the appearance of a fluorescent metabolite in the phloem in relation to the induction of resistance against C. lagenarium after initial infection with TNV (A and C) or with C. lagenarium (B and D). At time zero the first leaves of cucumber plants were inoculated either with TNV [a homogenate of cucumber leaves infected with TNV was gently rubbed onto the surface of the leaf with a thick, short-haired paintbrush; mock-inoculated controls were treated in strictly the same way with a homogenate from healthy cucumber leaves (17)] or with C. lagenarium (5 μL of a suspension of 200,000 spores per milliliter, dispersed in 25 droplets over the surface of the first leaf). (A and B) At each time point, two cucumber plants were cut with a razor blade through the stem above the first and below the second leaf. We harvested the phloem by collecting the exudate into ice-cold 50% aqueous methanol (v/v) and pooled samples for analysis by HPLC. Methanolic extracts were injected onto a C18 octadecylsilylene, 5-μm column equilibrated with 13% (v/v) buffered acetonitrile (sodium acetate buffer, 50 mM, pH 4.5). Elution at 2 ml/min was programmed as a linear gradient of 13 to 35% buffered acetonitrile, with the gradient started 16 min after injection. Peak detection was by fluorescence (excitation, 290 nm; emission, 402 nm). The data were plotted as the concentration of salicylic acid in phloem. (C and D) At each time point, three inoculated cucumber plants were challenged with a secondary inoculation of C. lagenarium on leaf 2. The number of lesions on the challenged leaf were plotted as a function of the day of challenge inoculation (error limits are ±SD, n = 3).
Evidence that SAR requires SA

![Diagram of the biochemical degradation of naphthalene by P. putida strain PpG7](image)

**Fig. 1.** Function of nahG. Biochemical degradation of naphthalene by *P. putida* strain PpG7 occurs in 13 steps encoded by the bacterial *nah* genes (12). The letters A to M represent the enzymes encoded by the genes *nahA* to *nahM*. The *nahG* gene encodes salicylate hydroxylase that uses NADH (reduced form of nicotinamide adenine dinucleotide) as a cofactor to decarboxylate and hydroxylate SA to catechol.

**Table 1.** Salicylic acid and TMV lesion size in buffer-pretreated and TMV-pretreated tobacco plants. Salicylic acid is expressed as nanograms of SA per gram of extracted tissue. The values are the means ± SD after correction for recovery during the extraction (57.1%) and are based on triplicate assays, except for the NahG-1 mock-inoculated sample which was based on a duplicate assay. The sizes of TMV lesions are given in millimeters. The numbers are the mean ± SD. We measured 90 lesions from three to five plants per line. The data were statistically analyzed by analysis of variance (ANOVA II) and then by a Tukey-Kramer test. Within each treatment category, statistically equivalent groups (*P* ≥ 0.05) are indicated by letters a through d.

<table>
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<tr>
<th>Line</th>
<th>Salicylic acid (ng/g)</th>
<th>Fold induction*</th>
<th>TMV lesion size ± SD (mm)</th>
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<td>3.8 ± 0.4 (c)</td>
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<td>NahG-10</td>
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</table>

*Expressed as amount of SA in TMV-inoculated tissue, divided by amount of SA in mock-inoculated tissue.
Figure 1. Schematic Diagram of the Grafting Experiments Using Xanthi and NahG Plants.

The rootstocks of the grafted plants were either TMV or mock treated ("induction"), followed by a challenge inoculation of the scion tissue 7 days later. The levels of infection in the challenged leaves were determined 5 or more days after challenge inoculation. X, Xanthi-nc; N, NahG.

Table 1. Induction of Resistance to TMV in Grafted Tobacco Plants by TMV Pretreatment

<table>
<thead>
<tr>
<th>Graft</th>
<th>Inducer</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
<th>Experiment 4</th>
<th>Average %</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>Mock</td>
<td>2.70 ± 0.46</td>
<td>0.82 ± 0.13</td>
<td>0.90 ± 0.20</td>
<td>1.56 ± 0.18</td>
<td>41%</td>
</tr>
<tr>
<td>X</td>
<td>TMV</td>
<td>1.40 ± 0.64  (52%)</td>
<td>0.34 ± 0.03  (37%)</td>
<td>0.48 ± 0.40  (53%)</td>
<td>0.31 ± 0.11  (20%)</td>
<td>41%</td>
</tr>
<tr>
<td>N</td>
<td>Mock</td>
<td>3.10 ± 0.43</td>
<td>1.94 ± 0.27</td>
<td>1.70 ± 0.40</td>
<td>2.19 ± 0.27</td>
<td>99%</td>
</tr>
<tr>
<td>N</td>
<td>TMV</td>
<td>3.19 ± 0.53  (103%)</td>
<td>1.61 ± 0.22  (89%)</td>
<td>1.76 ± 0.26  (104%)</td>
<td>2.32 ± 0.23  (106%)</td>
<td>99%</td>
</tr>
<tr>
<td>N</td>
<td>Mock</td>
<td>2.75 ± 0.40</td>
<td>1.92 ± 0.19</td>
<td>1.20 ± 0.40</td>
<td>2.17 ± 0.16</td>
<td>99%</td>
</tr>
<tr>
<td>X</td>
<td>TMV</td>
<td>3.06 ± 0.54  (111%)</td>
<td>1.63 ± 0.17  (85%)</td>
<td>1.55 ± 0.40  (120%)</td>
<td>2.20 ± 0.22  (101%)</td>
<td>104%</td>
</tr>
<tr>
<td>X</td>
<td>Mock</td>
<td>2.08 ± 0.48</td>
<td>1.92 ± 0.07</td>
<td>0.93 ± 0.20</td>
<td>1.38 ± 0.36</td>
<td>40%</td>
</tr>
<tr>
<td>N</td>
<td>TMV</td>
<td>1.19 ± 0.56  (57%)</td>
<td>0.41 ± 0.02  (21%)</td>
<td>0.47 ± 0.18  (51%)</td>
<td>0.44 ± 0.18  (32%)</td>
<td>40%</td>
</tr>
</tbody>
</table>

Grafted plants are indicated in the table (X, Xanthi-nc; N, NahG; scion above, rootstock below the dividing line). Seven days after rootstock-inducing inoculation (mock or TMV), scion leaves were assayed for resistance by a TMV challenge. The lesion size (average diameter ± sd in millimeters) 5 to 10 days postchallenge from four independent experiments are shown. The lesions on the TMV-induced plants are expressed relative to the mock pretreated plants for each experiment and averaged over the four experiments (average %).
Pathogenesis-related (PR) gene expression is associated with SAR

Use of PR1 as a reporter gene to isolate Arabidopsis lines with mutations in the SAR pathway . . .

Ward et al. 1991
The Arabidopsis NPR1 Gene That Controls Systemic Acquired Resistance Encodes a Novel Protein Containing Ankyrin Repeats

Hui Cao, Jane Glazebrook, Joseph D. Clarke, Sigrid Volko, and Xinnian Dong

Figure 2. Phenotypic Analysis of Complementation

(A) Northern blot analysis of expression of the PR-1 gene in wild type (Col-0, lanes 1–3), npr1-2 (lanes 4–6), and npr1-2 transformants with a noncomplementing cosmid (m305-2-7, lanes 7–9) and with complementing cosmids (21A4-P5-1, lanes 10–12, and 21A4-6-1, lanes 13–15). RNA samples were prepared from 15-day-old seedlings grown on MS media (lanes 1, 4, 7, 10, and 13), MS with 0.1 mM INA (lanes 2, 5, 8, 11, and 14), and MS with 0.1 mM SA (lanes 3, 6, 9, 12, and 15).

(B) Disease symptoms (top panels) and BGL2-GUS expression (bottom panels) induced by Psm ES4326 on wild type (left panels), npr1-1 (middle panels), and an npr1-1 transformant with a complementing cosmid (21A4-4-3-1, right panels). Psm ES4326 suspension (OD600 = 0.001, the leaf samples on the left; OD600 = 0.0001, the leaf samples on the right) was infiltrated into the left side of the leaves and symptoms were examined 3 days after infection. GUS staining was carried out according to Jefferson et al. 1987.
Nuclear Localization of NPR1 Is Required for Activation of PR Gene Expression
Mark Kinkema, Weihua Fan, and Xinnian Dong

Figure 1. Complementation of the npr1 Mutant Phenotypes by the NPR1-GFP Fusion Protein.

(A) Gel blot of RNA (20 µg) from wild type (W.T.), npr1, two independent 35S::NPR1-GFP transformants (NPR1-GFP; in npr1), and a 35S::GFP transformant (GFP; in npr1).

(B) Gel blot of protein (100 µg) from wild type, two independent 35S::NPR1-GFP transformants (in npr1), and a 35S::GFP transformant (in npr1).

(C) BGL2::GUS expression in wild type, npr1, and two independent 35S::NPR1-GFP transformants (in npr1) grown for 13 days on MS medium with or without 0.1 mM INA.

(D) Growth of wild type, npr1, a 35S::NPR1-GFP transformant (in npr1), and a 35S::GFP transformant (in npr1) on MS medium containing 0.5 mM SA.

(E) Symptoms on leaves infected with Psm ES4326. The left halves of leaves from 4-week-old plants were infected with Psm ES4326 (OD600 = 0.0001).

Figure 2. Nuclear Localization of NPR1-GFP in Response to SAR Induction.

(A) Confocal images of GFP fluorescence in mesophyll cells (top and bottom pairs of images) and in guard cells (middle pair of images) of cotyledons from 7-day-old seedlings grown on MS or MS-INA. For mesophyll cells, GFP fluorescence is shown in the green channel and differential interference contrast images are shown in the red channel.

(B) Gel blot of total protein (100 µg) and the nuclear-fractionated protein (12 µg). Protein was isolated from transgenic seedlings 35S::NPR1-GFP or 35S::npr157-GFP (NPR1-GFP or npr157-GFP) grown for 12 days on MS medium containing either 0.1 or 0.3 mM SA. The blot was probed with antibodies against GFP. The cytoplasmic npr157-GFP protein served as a control, indicating that the nuclear fraction contains relatively little cytoplasmic contamination.

Figure 4. Increased Nuclear Accumulation of NPR1-GFP Correlates with Increased Expression of PR Genes.

(A) Representative images of nuclear NPR1-GFP fluorescence in leaf mesophyll cells of seedlings grown for 12 days on MS medium containing either 0.1 or 0.3 mM SA.

(B) Gel blot of total protein (40 µg) and the nuclear-fractionated protein (20 µg). Protein was isolated from transgenic seedlings 35S::NPR1-GFP or 35S::npr157-GFP (NPR1-GFP or npr157-GFP) grown for 12 days on MS medium containing either 0.1 or 0.3 mM SA. The blot was probed with antibodies against GFP. The cytoplasmic npr157-GFP protein served as a control, indicating that the nuclear fraction contains relatively little cytoplasmic contamination.

(C) Gel blot of RNA from npr1 and an npr1 line expressing NPR1-GFP. Seedlings were grown for 12 days on MS medium containing either 0.1 or 0.3 mM SA. The blot was probed for NPR1, PR-1, and 18S rRNA.

(D) GFP fluorescence and BGL2::GUS expression in a 35S::NPR1-GFP transformant (in npr1) after infiltration with either Psm ES4326 (OD600 = 0.001 in 10 mM MgCl2) or 10 mM MgCl2. Leaves were infected o
Nuclear Localization of NPR1 Is Required for Activation of PR Gene Expression

**Figure 4.** Increased Nuclear Accumulation of NPR1-GFP Correlates with Increased Expression of PR Genes.

(A) Representative images of nuclear NPR1-GFP fluorescence in leaf mesophyll cells of seedlings grown for 12 days on MS medium containing either 0.1 or 0.3 mM SA.

(B) Gel blot of total protein (40 µg) and the nuclear-fractionated protein (20 µg). Protein was isolated from transgenic seedlings 35S::NPR1-GFP or 35S::npr157-GFP (NPR1-GFP or npr157-GFP) grown for 12 days on MS medium containing either 0.1 or 0.3 mM SA. The blot was probed with antibodies against GFP. The cytoplasmic npr157-GFP protein served as a control, indicating that the nuclear fraction contains relatively little cytoplasmic contamination.

(C) Gel blot of RNA from npr1 and an npr1 line expressing NPR1-GFP. Seedlings were grown for 12 days on MS medium containing either 0.1 or 0.3 mM SA. The blot was probed for NPR1, PR-1, and 18S rRNA.

(D) GFP fluorescence and BGL2::GUS expression in a 35S::NPR1-GFP transformant (in npr1) after infiltration with either Psm ES4326 (OD600 = 0.001 in 10 mM MgCl2) or 10 mM MgCl2. Leaves were infected o

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**Figure 5.** Nuclear Localization of NPR1 Is Essential for Its Function in Activating PR-1 Gene Expression.

(A) Strategy used to control the nuclear localization of NPR1.

(B) Gel blot of RNA (10 µg) from 35S::NPR1 (in npr1) and three independent 35S::NPR1-HBD transformants (in npr1). Seedlings were grown for 14 days on MS medium with (+) or without (-) DEX (5 µM) and with or without INA (20 µM). The blot was probed for PR-1 and ubiquitin (UBQ) mRNA.
In Vivo Interaction between NPR1 and Transcription Factor TGA2 Leads to Salicylic Acid–Mediated Gene Activation in Arabidopsis

Weihua Fan and Xinnian Dong

Plant Cell, Vol. 14, 1377-1389, 002

In Vivo Interaction between NPR1 and Transcription Factor TGA2 Leads to Salicylic Acid–Mediated Gene Activation in Arabidopsis

Weihua Fan and Xinnian Dong

Figure 2. Overexpression of TGA2CT in Wild-Type Plants Results in an npr1-Like Phenotype.

(A) Protein gel blot analysis of the TGA2CT protein in 35S::TGA2CT transformants. Protein extracts were made from 2-week-old plants. Independent 35S::TGA2CT transformants (5, 6, 17, and 18) in the wild-type background (35S::TGA2CT [WT]) were compared with untransformed wild-type plants (WT). The His6-tagged TGA2CT was detected using an antibody against the His tag.

(B) RNA gel blot analysis of PR-1 gene expression. Plants were grown for 2 weeks on MS medium with (+) or without (-) 20 µM INA. Total RNA (10 µg) was used to make the blot, which was probed for the PR-1 and UBQ5 mRNAs.

(C) Disease symptoms caused by P. syringae pv maculicola ES4326. Leaves from 4-week-old wild-type, 35S::TGA2CT (WT) (line 17), and npr1-2 plants were infiltrated with a P. syringae pv maculicola ES4326 suspension of OD600 = 0.0001. Photographs were taken of representative leaves 4 days after infection.

(D) Growth of plants on MS medium containing 0.5 mM SA. The photographs were taken 7 days after germination.
Interplay of signaling pathways in plant disease resistance

Bart J. Feys and Jane E. Parker

FIGURE 1. Overview of local and systemic signaling in Arabidopsis disease resistance. A number of resistance pathways (SAR, ISR and plant defensin induction), with different requirements for the signaling molecules salicylic acid (SA), jasmonic acid (JA) and ethylene, and that lead to the induction of different sets of defense-related genes, have been defined in Arabidopsis. Recruitment of NPR1 to these pathways appears to depend on the nature of the pathogen input signal and evidence points to NPR1 functioning as signal modulator, determining a particular output. NPR1 function in SAR requires the removal of a negative control by SNI1. Analysis of several resistance-upregulating mutations (cpr5, cpr6 and ssi1) also points to cross-talk between SA- and JA/ethylene-responsive processes. Non-pathogenic rhizobacteria induce a JA/ethylene-dependent, SA-independent systemic response (ISR) that requires functional NPR1 but induces an as yet undefined set of downstream events. Genes that are known to be required for pathogen-induced SA accumulation and full resistance are shown, as well as signaling components from the JA and ethylene pathways that control pathogen-induced defensin synthesis and establishment of ISR.
R proteins differ in their requirements for EDS1 And NDR1

![Diagram of R proteins signaling pathways]

Figure 3. Dissection of R gene-mediated signaling pathways in Arabidopsis. Genetic analysis of NB-LRR type R genes in Arabidopsis has shown that R proteins with an amino-terminal TIR domain predominantly signal through EDS1, whereas LZ-containing R proteins require NDR1 and PBS2 to initiate defense responses. Interestingly, RPP8, an LZ-NB-LRR type R protein, functions independently of these two pathways. RPP7, an R gene that has not yet been cloned, confers isolate-specific resistance to P. parasitica through a novel pathway that may or may not be shared with RPP8 ([24]). Mutations in PAD4 cause a partial loss of RPP5-mediated resistance to P. parasitica in contrast to the eds1 mutant, which shows a full loss of RPP5 function. Both EDS1 and PAD4 operate upstream of pathogen-induced SA accumulation[28] (M.A. Newman and J.E. Parker, unpublished data), which in turn is defined by the genes SID1/EDS5 and SID2. NPR1 is a key modulator of SA responses that functions downstream of SA perception[34]. Mutations in PBS3 affect R genes of both the TIR and LZ class and have therefore been placed at a point of convergence between both pathways. The effect of the pbs3 mutation on SA levels, as well as its position relative to NPR1, is unknown. The requirement for PBS1 was shown to be specific for RPS5.
Table 2: Signaling components that affect multiple *R* gene-mediated pathways.

<table>
<thead>
<tr>
<th><em>R</em> gene</th>
<th>EDS1</th>
<th>PAD4</th>
<th>NDR1</th>
<th>PBS2/RAR1</th>
<th>SGT1</th>
<th>NPR1/NIM1</th>
<th>PBS3</th>
<th>RAR2</th>
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<tr>
<td><em>RPM1</em> (CC)</td>
<td><em>No (1, 58)</em></td>
<td>No (58)</td>
<td>Yes (30)</td>
<td>Yes (121, 196)</td>
<td>No (4, 178)</td>
<td>No (140)</td>
<td>Yes (196)</td>
<td></td>
</tr>
<tr>
<td><em>RPS2</em> (CC)</td>
<td>Y/N (1, 58)</td>
<td>N/Y (58)</td>
<td>Yes (30)</td>
<td>Yes (121)</td>
<td>No (4)</td>
<td>No (140)</td>
<td>Yes (196)</td>
<td></td>
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<tr>
<td><em>RPS4</em> (TIR)</td>
<td>Yes (58)</td>
<td>Y/N (58)</td>
<td>No (1)</td>
<td>Yes (121)</td>
<td>No (4, 178)</td>
<td>No (140)</td>
<td>Yes (196)</td>
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<tr>
<td><em>RPS5</em> (CC)</td>
<td>No (1)</td>
<td>Yes (30)</td>
<td>Yes (121, 196)</td>
<td>No (178)</td>
<td>Yes (196)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>RPP1A</em> (TIR)</td>
<td>Yes (58)</td>
<td>Y/N (58)</td>
<td>No (52)</td>
<td>No (121)</td>
<td>No (4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>RPP1B</em> (TIR)</td>
<td>Yes (58)</td>
<td>Y/N (58)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td><em>RPP1C</em> (TIR)</td>
<td>Yes (58, 131)</td>
<td>Y/N (58)</td>
<td>No (1)</td>
<td>No (121, 196)</td>
<td>Yes (4, 178)</td>
<td>No (196)</td>
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<td>Yes (1)</td>
<td></td>
<td>No (1)</td>
<td>No (121, 196)</td>
<td>Yes (4, 178)</td>
<td></td>
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<td><em>RPP4</em> (TIR)</td>
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<td>Yes (183)</td>
<td>Y/N (1, 183)</td>
<td>Yes (121, 183, 196)</td>
<td>Yes (4, 178)</td>
<td>Y/N (183)</td>
<td>Y/N (183, 196)</td>
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<td><em>RPP5</em> (TIR)</td>
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<td>Y/N (58)</td>
<td>Y/N (1, 183)</td>
<td>Yes (121)</td>
<td>Yes (4)</td>
<td>Yes (140)</td>
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<td><em>RPP6</em></td>
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<td></td>
<td>No (196)</td>
<td>Yes (178)</td>
<td>Yes (196)</td>
<td></td>
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<tr>
<td><em>RPP7</em> (CC)</td>
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<td>No (58)</td>
<td></td>
<td>No (121)</td>
<td>No (4)</td>
<td>No (140)</td>
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<td>14 (178)</td>
<td>No (196)</td>
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<tr>
<td><em>RPP8</em> (CC)</td>
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<td>No (58)</td>
<td>No (1)</td>
<td>No (121)</td>
<td>No (4)</td>
<td>No (140)</td>
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<td>No (52)</td>
<td></td>
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<td><em>RPP12</em></td>
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<td>No (15)</td>
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<td>No (15)</td>
<td>No (15)</td>
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<td>No (52)</td>
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<td><em>RPP19</em></td>
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<td>Yes (1, 58)</td>
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<td>No (1)</td>
<td>Yes (121)</td>
<td>Y/N (4)</td>
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<tr>
<td><em>RPW8</em> (CC-like)</td>
<td>Y (200)</td>
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<td>Yes (102)</td>
<td>Yes (103)</td>
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<td><em>N</em> (TIR)</td>
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</table>
A model describing the positions of Arabidopsis genes in signal transduction networks that control the activation of defense responses. This figure is meant to provide a rough outline of the sites of action of various gene products in disease resistance signaling. It does not account for all of the described phenotypes of the various signaling mutants. Boxes containing the word 'resistance' represent the numerous effectors of resistance, whose effectiveness may vary greatly according to the pathogen eliciting the response. There are certainly interconnections among the pathways shown in (a), (b), (c), and (d), but many of these are not sufficiently clear to allow them to be easily drawn.

(a) Three R-gene dependent pathways are shown, one that requires NDR1 and PBS2, a second that requires EDS1 and PAD4, and a third for which the required genes have not been reported. (b) In SA-dependent signaling, CPR1, EDR1, and CPR5 tend to repress pathway activation, and act upstream of PAD4. The cpr6 mutation exerts a positive effect upstream of PAD4, but it is difficult to predict the function of the wild-type gene as cpr6 is dominant. PAD4 and EDS1 promote SA accumulation, as do SID2 and EDS5. SA also promotes expression of PAD4 and EDS1. PAD4 promotes expression of resistance responses in an SA-independent manner. Formation of lesions promotes SA accumulation in a manner that is independent of PAD4 and EDS1, but may require EDS5. Downstream of SA, NPR1 mediates the activation of expression of genes such as PR-1, acting together with TGA transcription factors in a manner that is inhibited by SN1. DTH9 also acts downstream of SA to activate resistance responses. (c) JA and ET act together to activate expression of genes such as PDF1.2. This activation is promoted by lesion formation and by cpr6. MPK4, COI1, and JAR1 are required to transduce the JA signal, while EIN2 is required to transduce the ET signal. Camalexin production requires both PAD4 and COI1, and some lesion-mimic mutations cause camalexin accumulation in lesions. (d) ISR activation requires JA, followed by ET. ISR1 affects sensitivity to ET.

NPR1 is required downstream of JA and ET to activate resistance. The model does not show the complicated interactions between SA and JA signaling. For example, MPK4 inhibits SA signaling, possibly by promoting JA signaling, but the nature of the relationship between the promotion of JA signaling and the inhibition of SA signaling is not understood.

Glazebrook 2001