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NPR1: the spider in the web of induced resistance signaling pathways

Corné MJ Pieterse and LC Van Loon

The plant hormones salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) are major players in the regulation of signaling networks that are involved in induced defense responses against pathogens and insects. During the past two years, significant progress has been made in understanding the function of NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES1 (NPR1), a key regulator of systemic acquired resistance (SAR), that is essential for transducing the SA signal to activate *PATHOGENESIS-RELATED* (*PR*) gene expression. SA-mediated redox changes in *Arabidopsis* cells regulate both the functioning of NPR1 and its binding to TGA1, a member of the TGA family of transcription factors that activate SA-responsive elements in the promoters of *PR* genes upon binding with NPR1. Apart from its role in regulating SAR in the nucleus, a novel cytosolic function of NPR1 in cross-communication between SA- and JA-dependent defense signaling pathways has been identified. Other advances in induced resistance signaling, such as the implication that ET is involved in the generation of systemic signal molecules, the suggestion of the involvement of lipid-derived molecules in long-distance signaling, and the identification of new components of various systemic defense signaling pathways, shed new light on how plants actively defend themselves against harmful organisms.

Addresses
Section Phytopathology, Faculty of Biology, Utrecht University, PO Box 80084, 3508 TB Utrecht, The Netherlands
e-mail: c.m.j.pieterse@bio.uu.nl

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Abbreviations

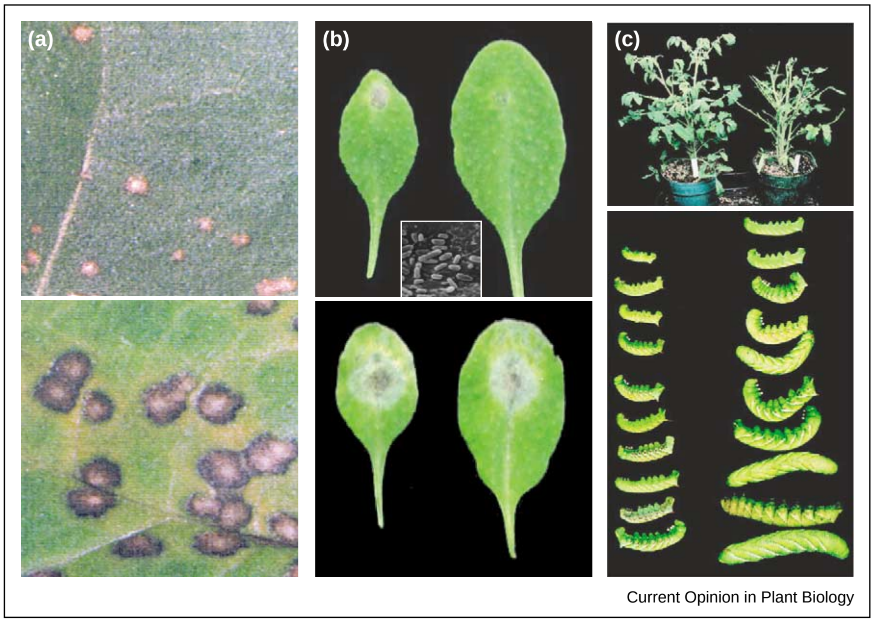
coi1	*coronatine insensitive1*
DIR1	*DEFECTIVE IN INDUCED RESISTANCE1*
ERF1	ETHYLENE RESPONSE FACTOR1
ET	ethylene
ISR	induced systemic resistance
JA	jasmonic acid
nahG	salicylate hydroxylase gene
NIM1	NON-INDUCIBLE IMMUNITY1
NPR1	NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES1
PI	proteinase inhibitor
PR	*PATHOGENESIS-RELATED*
ROI	reactive oxygen intermediates
SA	salicylic acid
SABP2	SA-BINDING PROTEIN2
SAR	systemic acquired resistance
SCF^{COI1}	SKP1, CDC53p/CUL1 F-box^{COI1}
TMV	Tobacco mosaic virus

Introduction

Plant innate immunity is based on a surprisingly complex response that is highly flexible in its capacity to recognize and counteract different invaders. To combat invasion by microbial pathogens and herbivorous insects effectively, plants make use of pre-existing physical and chemical barriers, as well as inducible defense mechanisms that become activated upon attack. Apart from reacting locally, plants can mount a systemic response that establishes an enhanced defensive capacity in tissues distant from the site of primary attack. This systemically induced response protects the plant against subsequent invaders. Several biologically induced systemic defense responses have been characterized in detail. These include systemic acquired resistance (SAR), which is triggered by necrotizing pathogens [1]; induced systemic resistance (ISR), which is activated upon colonization of roots by selected strains of non-pathogenic rhizobacteria [2]; and wound-induced defense, which is typically elicited upon tissue damage such as that caused by feeding insects ([3]; Figure 1). Induced defense responses are regulated by a network of interconnecting signal transduction pathways in which the hormonal signals salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) play a major role [4–6], and other hormones such as brassinosteroids and abscisic acid can also be involved [7–9].

SA, JA, and ET accumulate in response to pathogen infection or herbivore damage, leading to the activation of distinct and partly overlapping sets of defense-related genes. Global expression profiling of *Arabidopsis* wildtype plants and several *Arabidopsis* SA-, JA-, or ET-signaling mutants that were infected by *Pseudomonas syringae* pv. *maculicola* revealed substantial cross-talk between the signaling pathways induced by the three hormones [10•]. It has become clear that different defensive pathways are differentially effective against specific types of attackers. In general, pathogens that have a biotrophic lifestyle are more sensitive to SA-dependent responses, whereas usually necrotrophic pathogens and herbivorous insects are better resisted by JA/ET-dependent defenses [11,12,13•]. For instance, the activation of SA-dependent

Figure 1



Effects of biologically induced systemic defense responses on pathogen and insect resistance. **(a)** Pathogen-induced SAR in tobacco against TMV. Inoculation of tobacco cv. Samsun *NN* with TMV induces the formation of lesions as a result of a hypersensitive response (lower panel). A signal is generated and systemically transported throughout the plant, leading to a SA-dependent defense response that is effective against a broad spectrum of pathogens. Subsequent inoculation of uninfected plant parts with TMV results in the formation of lesions that are significantly reduced in size (upper panel) compared to those on the uninduced leaves. **(b)** Rhizobacteria-ISR in *Arabidopsis thaliana* against the fungal pathogen *Alternaria brassicicola*. Plants whose roots are colonized by selected rhizobacterial strains of *P. fluorescens* systemically trigger a JA/ET-dependent defense response in foliar tissues that, like SAR, is effective against a broad spectrum of plant pathogens. Upon inoculation with *A. brassicicola*, ISR-expressing plants (upper panel) develop significantly less-severe symptoms compared to non-induced plants (lower panel). The insert in the upper panel shows an electron micrograph of *P. fluorescens* bacteria on the surface of a plant root. **(c)** Wound/herbivore-induced resistance in tomato against tobacco hornworm larvae. (Upper panel) On the left, a wildtype tomato plant at the end of a feeding trial. On the right, the tomato mutant *suppressor of prosystemin-mediated responses2* (*spr2*), which is affected in the *SPR2* gene and is incapable of producing a systemic wound signal, resulting in compromised defense against feeding insects. (Lower panel) The hornworm larvae recovered from the wildtype and mutant plants. The wound response is regulated by a JA-dependent signaling pathway. Photographs in panel (c) were kindly provided by Greg Howe, Michigan State University, and reproduced from [11] with permission.

SAR by avirulent *P. syringae* pv. *tomato* resulted in a significant level of protection against the biotrophic pathogen Turnip crinkle virus. In contrast, JA/ET-dependent ISR, triggered by non-pathogenic *Pseudomonas fluorescens* rhizobacteria, was ineffective against the virus [13•]. Conversely, rhizobacteria-mediated ISR provided significant protection against the necrotrophic fungus *Alternaria brassicicola*, whereas pathogen-induced SAR was ineffective. Thus, plants are able to differentially activate defense responses depending on the (micro)organism perceived. Cross-communication between defense pathways can provide a regulatory potential that allows the plant to fine-tune its defense responses, depending on which attacker it is encountering.

In this review, we discuss new developments in induced defense signaling that have emerged during the past two years. We emphasize the central role of the regulatory protein NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES1 (NPR1). A complete overview of the current status of induced resistance is beyond the scope of this short update.

Systemic signaling

SAR is by far the best-studied induced resistance response. The onset of SAR is accompanied by a local and systemic increase in endogenous levels of SA. Although SA moves through the plant, it is not the mobile signal for SAR [1]. Analysis of an *Arabidopsis* T-DNA insertion line identified the *DEFECTIVE IN INDUCED RESISTANCE1* (*DIR1*) gene, which encodes a putative apoplastic lipid-transfer protein that is required for pathogen-induced SAR [14•]. Assessment of the ability of petiole exudates from wildtype and *dir1* plants to induce SAR-related gene expression indicated that *dir1* mutant plants are incapable of either producing or transmitting the mobile signal that is essential for the systemic expression of SAR. Maldonado *et al.* [14•] suggest that DIR1 interacts with a lipid-derived molecule to allow long-distance signaling. Interestingly, SA-BINDING

PROTEIN2 (SABP2) of tobacco, which functions as a receptor for SA in Tobacco mosaic virus (TMV)-infected tobacco, is a lipase whose activity is stimulated by SA binding and that may generate a lipid-derived signal that functions in SAR [15•]. Moreover, SUPPRESSOR OF FATTY ACID DESATURASE DEFICIENCY1 (SFD1), which is required for the systemic activation of SAR also appears to be involved in lipid metabolism [16]. Together, these findings suggest that lipid-derived signals are important components of long-distance signaling in SAR.

By using reciprocal grafts of wildtype tobacco plants and ethylene-insensitive (Tetr) tobacco plants that express a mutant copy of the *Arabidopsis ETHYLENE RESPONSE1* (*ETR1*) gene, Verberne and coworkers [17•] demonstrated that ET is also required for the production or transmission of the systemic SAR signal in TMV-infected leaves. ET-insensitive scions that were grafted onto TMV-infected wildtype rootstocks were capable of mounting SAR. However, TMV-infected rootstocks of ET-insensitive tobacco were unable to systemically trigger SA accumulation, *PATHOGENESIS-RELATED* (*PR*) gene expression or SAR in wildtype scions. Interestingly, ET signaling is similarly implicated in the generation/transmission of the mobile signal involved in the JA/ET-dependent ISR that is activated by non-pathogenic rhizobacteria [18]. Although the signaling pathways that are involved in SAR and ISR seem to be distinct [2], it is tempting to speculate that the synthesis or transmission of their mobile long-distance signals requires similar ET-dependent processes.

Significant progress has also been made in elucidating the mechanisms involved in wound- and herbivore-induced signaling in tomato, which are associated with the systemic activation of genes that encode defensive proteinase inhibitors (PIs). The 18-amino-acid peptide SYSTEMIN, which is cleaved off from PROSYSTEMIN upon wounding, acts as a mobile signal that initiates the JA biosynthesis that is required for the activation of *PI* genes [19]. By using reciprocal grafts and tomato mutants that are affected in either JA biosynthesis or action, Howe and coworkers refined this model [11,20,21•]. They showed that although (PRO)SYSTEMIN is required for the wound-induced biosynthesis of JA, it is not the mobile long-distance signal that is required for the systemic activation of *PI* genes. Rather, (PRO)SYSTEMIN must act at or near the site of wounding to increase JA biosynthesis to a level required for the production of the systemically transported signal. The recognition and transduction of the long-distance signal results in enhanced production of JA and subsequent *PI* gene expression, a process that again depends on JA signaling. Howe and coworkers [11,20,21•] postulated that JA itself, or a related compound from the octadecanoid pathway, may act as the transmissible wound signal.

SAR signal transduction

SA accumulates in non-infected plant tissues that perceive the long-distance SAR signal, resulting in the upregulation of a large set of defensive genes, including those that encode PR proteins [1,22]. Besides the direct activation of SA-responsive *PR* genes, SAR is also associated with an ability to induce cellular defense responses more rapidly or to a greater degree than in non-induced plants. This process, called ‘priming’ [23], leads to the enhanced expression of defense-related genes once pathogen infection occurs. Compelling evidence for the essential role of SA in SAR was originally provided through the use of transgenic plants that expressed the bacterial salicylate hydroxylase gene *nahG* [24]. Two studies recently demonstrated, however, that the *nahG* transgene can have pleiotropic effects on defense signaling that cannot be attributed to the low SA content of *nahG* plants [25,26]. Thus, data from experiments that use *NahG* plants should be interpreted with caution. Mutants in which SA biosynthesis or action is disturbed, such as those mutated in the SA-biosynthesis gene *SA INDUCTION-DEFICIENT2* (*SID2*; which encodes isochorismate synthase [27,28]) or in *ENHANCED DISEASE SUSCEPTIBILITY5* (*EDS5*; which encodes a membrane protein with homology to bacterial multidrug and toxin extrusion [MATE] transporters [27,29]) will be increasingly instrumental in unraveling the role of SA in defense signaling.

Transduction of the SA signal to activate *PR* gene expression and SAR requires the function of NPR1, also known as NON-INDUCIBLE IMMUNITY1 (NIM1). NPR1 is a regulatory protein that was identified in *Arabidopsis* through several genetic screens for SAR-compromised mutants [30]. Upon induction of SAR, NPR1 is translocated into the nucleus [31]. NPR1 acts as a modulator of *PR* gene expression but does not bind DNA directly [32]. Yeast two-hybrid analyses have indicated that NPR1 acts through members of the TGA subclass of the basic leucine zipper (bZIP) family of transcription factors (TGAs), which are implicated in the activation of SA-responsive *PR* genes [32–34]. Electromobility shift assays showed that NPR1 substantially increases binding of TGA2 to SA-responsive promoter elements in the *Arabidopsis PR-1* gene [32], suggesting that NPR1-mediated DNA binding of TGAs is important for *PR* gene activation.

NPR1–TGA interactions *in vivo*

Compelling evidence that binding between NPR1 and TGAs occurs *in planta* has been provided by several studies. Subramaniam and coworkers [35] used a protein-fragment-complementation assay to demonstrate interactions between NPR1 and TGA2 *in vivo*, and showed that the SA-induced interaction is predominantly localized in the nucleus. Fan and Dong [36••] followed a genetic approach using *Arabidopsis* transgenics that overexpressed the carboxy-terminal domain of TGA2. This mutant TGA2 protein was capable of interacting with NPR1 but lacked the DNA-binding activity that is important for TGA function. Accumulation of this dominant-negative mutant TGA2 protein in a wildtype background led to the dose-dependent abolition of TGA function in an NPR1-dependent manner. The resulting phenotype resembled that of mutant *npr1* plants in that the ability to express *PR-1* in response to the SA analog 2,6-dichloroisonicotinic acid (INA) was impaired, and susceptibility to infection by *P. syringae* pv. *maculicola* was increased. Furthermore, evidence that NPR1–TGA interactions are essential for SA-mediated gene activation *in vivo* has been provided by both genetic and immunoprecipitation experiments. Using a chimeric reporter system in *Arabidopsis*, Fan and Dong [36••] showed that TGA2 activates the *in planta* expression of target reporter genes in response to SA in an NPR1-dependent manner. Chromatin immunoprecipitation experiments revealed that both TGA2 and TGA3 are recruited *in vivo* in a SA- and NPR1-dependent manner to SA-responsive elements in the *PR-1* promoter [37•]. The promoter occupancy of these TGAs was linked to the SA-induced onset of *PR-1* gene expression, supporting the notion that these transcription factors act as positive regulators of defense-related gene expression.

TGA function and redox regulation

Knockout analysis of single, double and triple mutants of *TGA2*, *TGA5* and *TGA6* in various combinations have established that these TGAs play an essential and partially redundant role in the activation of *PR* gene expression and SAR in *Arabidopsis* [38•]. Transgenic *Arabidopsis* plants that overexpressed *TGA5* possessed enhanced resistance towards the oomycete pathogen *Peronospora parasitica*, whereas this phenotype was not apparent for *TGA2* overexpressors [39]. This TGA5-mediated resistance was retained in the *nim1* mutant background, suggesting that TGA5 is also involved in the regulation of an SA-independent defense mechanism. Thus, different members of the TGA multigene family seem to make additional specific contributions to the regulation of defense responses.

The differential activities of TGAs may be regulated posttranscriptionally by distinct pathways that involve proteasome-mediated proteolysis [40]. Defense mechanisms are also regulated, however, at the level of NPR1 binding. The seven known *Arabidopsis* TGAs show differential binding activity towards NPR1 in yeast two-hybrid assays, with TGA2, TGA3 and TGA6 showing the strongest binding [32–34]. Interestingly, TGA1 and TGA4 do not bind to NPR1 in yeast assays. Using an *in-planta* transient expression assay that was mechanistically similar to the yeast two-hybrid system, however, Després and coworkers [41••] demonstrated that TGA1 does interact with NPR1 in *Arabidopsis* leaves upon SA treatment. In the same study, yeast two-hybrid assays of chimeric TGA1 proteins, in which the NPR1-interacting domain of TGA2 was swapped with the corresponding domain from TGA1, revealed that a 30-amino-acid segment is important for NPR1 interaction. Comparison of amino acid sequences with those of other TGAs revealed that both TGA1 and TGA4 contain two Cys residues in this 30-amino-acid region that are missing in the TGAs that interact with NPR1 in yeast. Site-directed mutation of these Cys residues to Asn and Ser transformed TGA1 and TGA4 into proteins that were capable of interacting with NPR1 in yeast. Because the Cys residues can form disulfide bridges that might prevent the interaction of TGA1 and TGA4 with NPR1, Després *et al.* [41••] tested whether the *in vivo* redox status of TGA1 affects NPR1 binding. Upon treatment of *Arabidopsis* leaves with SA, the Cys residues of TGA1 were reduced, thereby facilitating its interaction with NPR1 and subsequently enhancing the binding of TGA1 to SA-responsive promoter elements.

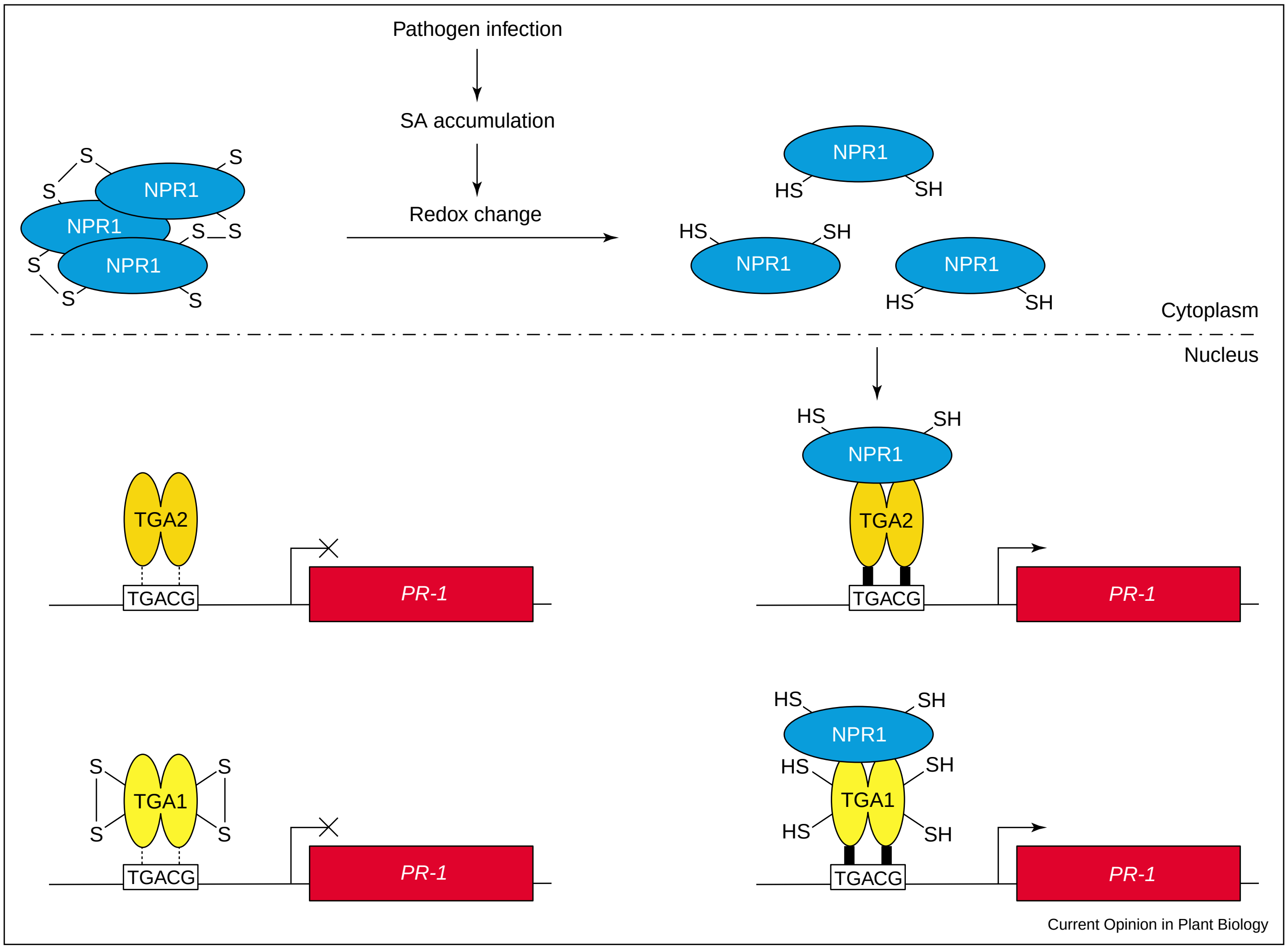
Redox changes connect the SA signal with NPR1 functioning

Clearly, NPR1 plays an important role in the SA-mediated activation of defense-related genes by enhancing the DNA binding of TGAs to SA-responsive elements in their promoters. But how does NPR1 transduce the SA signal? Previously, experiments with NPR1/NIM1 overexpressors demonstrated that high levels of NPR1 proteins *per se* do not induce *PR* expression or resistance, indicating that NPR1 needs to be activated by an unknown factor that acts downstream of SA [42,43]. The observations that NPR1 proteins from different plant species contain conserved Cys residues that are capable of forming inter- or intra-molecular disulfide bonds, and that a mutation in one of these Cys residues resulted in a mutant *npr1* phenotype, led Mou *et al.* [44••] to the hypothesis that NPR1 protein conformation may be sensitive to SA-induced changes in cellular redox status. In a series of elegant experiments, Mou *et al.* [44••] demonstrated that the induction of SAR is indeed associated with a change in redox state, possibly caused by the accumulation of antioxidants. Under these conditions, NPR1 was reduced from an inactive oligomeric complex to an active monomeric form. It seems that the monomeric form is required for *PR-1* activation, as inhibition of NPR1 reduction prevented *PR-1* gene expression. Mutation of two Cys residues that are crucial for NPR1 oligomer formation led to constitutive monomerization and nuclear localization of NPR1, and to constitutive expression of the *PR-1* gene. Thus, cellular redox changes that are induced as a result of SA accumulation connect the SA signal with NPR1 activity during SAR (Figure 2).

The cytosolic function of NPR1 in pathway cross-talk

Besides its crucial role in the regulation of *PR* gene expression, which is predominantly exerted in the

Figure 2

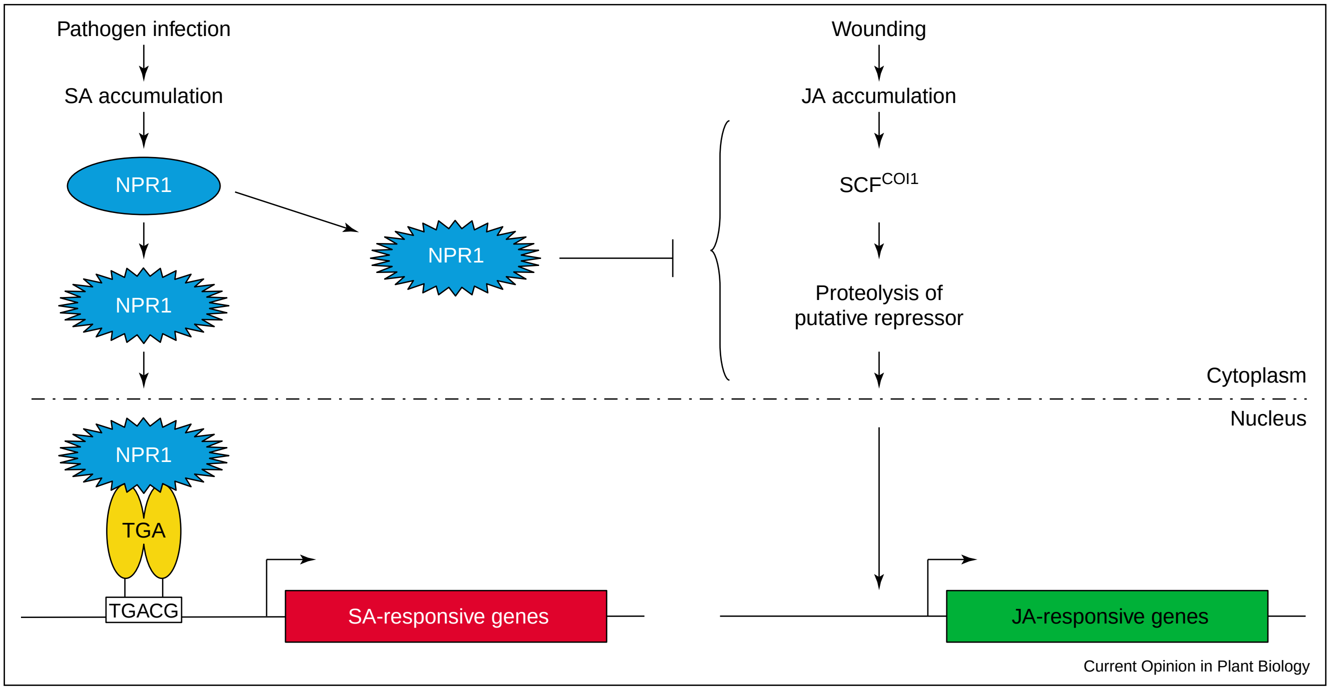


Model illustrating the role of SA-mediated redox changes, NPR1, and TGA transcription factors in SAR-related gene expression. In non-induced cells, oxidized NPR1 forms inactive oligomers that remain in the cytosol. Binding of TGAs to the cognate SA-responsive promoter elements (TGACG) (indicated by dotted lines) is not sufficient to activate the expression of *PR-1* genes. Upon infection by a necrotizing pathogen, SA accumulates and plant cells attain a more reducing environment, possibly because of the accumulation of antioxidants. Under these conditions, NPR1 is reduced from an inactive oligomeric complex to an active monomeric state through the reduction of intermolecular disulfide bonds. Monomeric NPR1 is then translocated into the nucleus where it interacts with TGAs, such as TGA2. The binding of NPR1 to TGAs stimulates the DNA-binding activity of these transcription factors to the cognate *cis* element (represented by black boxes), resulting in the activation of *PR-1* gene expression. In non-induced cells, TGAs that do not interact with NPR1 in yeast two-hybrid assays, such as TGA1, are oxidized and form intramolecular disulfide bridges that prevent interaction with NPR1. Upon accumulation of SA *in planta*, the change in redox status reduces the disulfide bonds in these TGAs, resulting in a conformational change that allows interaction with NPR1.

nucleus, an additional cytosolic function of NPR1 has been identified in the cross-talk between SA- and JA-dependent defense pathways. Activation of SAR suppresses JA signaling in plants, thereby prioritizing SA-dependent resistance over JA-dependent defenses [45]. Moreover, pharmacological and genetic experiments have shown that SA is a potent suppressor of JA-inducible gene expression [45]. Spoel *et al.* [46••] demonstrated that the antagonistic effect of SA on JA-triggered gene expression is negatively regulated through SA-activated NPR1. The nuclear localization of NPR1, which is essential for SA-mediated *PR* gene expression, appeared not to be required for the suppression of JA signaling. Thus, cross-talk between SA and JA is modulated through a novel function of NPR1 in the cytosol (Figure 3). The mode-of-action of NPR1 in the cytosol is unknown. It is tempting to speculate, however, that it interferes with the previously identified SCF^{COI1} ubiquitin-ligase complex [47,48] that regulates JA-responsive gene expression through targeted ubiquitination and subsequent proteasome-mediated degradation of a putative negative regulator of JA signaling.

Additional key elements that are involved in pathway cross-talk have been identified. For instance, the *Arabidopsis* transcription factor WRKY70 acts as both

Figure 3



Proposed model for cytosolic NPR1 as a modulator of cross-talk between SA- and JA-dependent defense responses. Infection by a necrotizing pathogen results in the accumulation of SA and the activation of NPR1. Activated NPR1 (represented by a star-shaped oval) is then translocated into the nucleus where it interacts with TGA transcription factors, ultimately leading to the activation of SA-responsive genes. The activation of NPR1 is controlled by SA-mediated redox changes in the cell (Figure 2). Wounding, such as that caused by feeding insects, results in the accumulation of JA. A putative repressor of JA-responsive gene expression is then ubiquitinated by a SCF^{COI1} ubiquitin-ligase complex that target proteins for degradation by the proteasome. Removal of the putative repressor protein results in the activation of JA-responsive genes. Inhibition of JA signaling by SA is regulated by a cytosolic function of SA-activated NPR1, but its site of action is not known.

an activator of SA-responsive genes and a repressor of JA-inducible genes, thereby integrating signals from these antagonistic pathways [49•]. In addition, the transcription factor ETHYLENE RESPONSE FACTOR1 (ERF1) integrates signals from the JA and ET pathways in activating defense-related genes that are responsive to both JA and ET [50•].

Conclusions

For many years, the mechanism by which SA accumulation activates NPR1 function in the SAR pathway was a major unknown. The discovery that SA-mediated changes in cellular redox status result in the reduction of inactive NPR1 oligomers to active monomers is a great step forward in our understanding of SAR signal transduction. The observation that a similar change in cellular redox status is essential for TGA1 to interact *in planta* with NPR1 indicates that perturbation of redox homeostasis by SA plays a dual role in SA signal transduction. It is tempting to speculate, therefore, that the cytosolic function of SA-activated NPR1 in modulating cross-talk between SA- and JA-dependent signaling is also redox regulated. How SA induces changes in the cellular redox status, and which redox mediators are involved, is largely unknown. Locally, pathogen attack results in increased SA levels and in the rapid production of reactive oxygen intermediates (ROI) and H_2O_2. To neutralize these potentially toxic compounds, ROI scavengers (such as the antioxidants catalase, superoxide dismutase and ascorbate peroxidase) are activated, thereby creating a shift toward reducing conditions in the plant cell [51–53]. Except for a single report [54], however, systemic activation of SAR in non-infected tissue has been associated neither with enhanced levels of ROI, nor with increases in antioxidant levels. In addition, Mou *et al.* [44••] were unable to demonstrate changes in redox status in leaves distal to the site of pathogen infection. Thus, the question of whether redox changes are involved in the SA-mediated, NPR1-dependent activation of *PR* genes in systemic tissue remains to be answered.

So which challenging questions remain to be addressed by future research on induced resistance signaling? First, NPR1 and its interacting partners are not the sole regulators of SA-responsive *PR* gene expression and SAR. Other essential transcription factors and their corresponding *cis*-acting elements have been identified, but their role in SAR still needs to be clarified. For instance, Desveaux *et al.* [55] identified a 'Whirly' transcription factor in *Arabidopsis* that is activated by SA but functions independently of NPR1 in activating SA-responsive gene expression and SAR, demonstrating that this type of induced resistance is regulated in a complex manner. Second, the identification of critical factors that are involved in the synthesis and transmission of the yet unidentified long-distance signals opens up new possibilities for discovering the nature of these mobile signals and their role in systemic induced resistance. Finally, global expression profiling has firmly established that induced defense responses in plants are regulated by a complex network of interconnecting signaling pathways. The molecular mechanisms by which plants utilize pathway cross-talk in fine-tuning their resistance response upon pathogen or insect attack is largely unknown, however, and identifying them is one of the exciting new challenges for the future.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Sticher L, Mauch-Mani B, Métraux J-P: **Systemic acquired resistance**. *Annu Rev Phytopathol* 1997, **35**:235-270.

2. Pieterse CMJ, Van Wees SCM, Ton J, Van Pelt JA, Van Loon LC: **Signalling in rhizobacteria-induced systemic resistance in *Arabidopsis thaliana***. *Plant Biol* 2002, **4**:535-544.

3. Kessler A, Baldwin IT: **Plant responses to insect herbivory: the emerging molecular analysis**. *Annu Rev Plant Biol* 2002, **53**:299-328.

4. Pieterse CMJ, Van Loon LC: **Salicylic acid-independent plant defence pathways**. *Trends Plant Sci* 1999, **4**:52-58.

5. Thomma BPHJ, Penninckx IAMA, Cammue BPA, Broekaert WF: **The complexity of disease signaling in *Arabidopsis***. *Curr Opin Immunol* 2001, **13**:63-68.

6. Glazebrook J: **Genes controlling expression of defense responses in *Arabidopsis* — 2001 status**. *Curr Opin Plant Biol* 2001, **4**:301-308.

7. Nakashita H, Yasuda M, Nitta T, Asami T, Fujioka S, Arai Y, Sekimata K, Takatsuto S, Yamaguchi I, Yoshida S: **Brassinosteroid functions in a broad range of disease resistance in tobacco and rice**. *Plant J* 2003, **33**:887-898.

8. Audenaert K, De Meyer GB, Höfte MM: **Abscisic acid determines basal susceptibility of tomato to *Botrytis cinerea* and suppresses salicylic acid-dependent signaling mechanisms**. *Plant Physiol* 2002, **128**:491-501.

9. Ton J, Mauch-Mani B: **β-amino-butyric acid-induced resistance against necrotrophic pathogens is based on ABA-dependent priming for callose**. *Plant J* 2004, **38**:119-130.

10. • Glazebrook J, Chen WJ, Estes B, Chang H-S, Nawrath C, Métraux J-P, Zhu T, Katagiri F: **Topology of the network integrating salicylate and jasmonate signal transduction derived from global expression phenotyping**. *Plant J* 2003, **34**:217-228.

A network of interconnecting signaling pathways regulates the response of plants to pathogen attack. The authors of this paper compared the transcript profiles of *Arabidopsis* wildtype plants and a set of SA-, JA-, or ET-signaling mutants after infection by *P. syringae*. Hierarchical clustering of co-regulated genes revealed the relationship between the respective signaling components in the defense-signaling network that is activated in *Arabidopsis* upon infection by *P. syringae*. The co-regulation and co-suppression of subgroups of genes in different arms of the signaling network demonstrated that there is extensive cross-talk between signaling pathways.

11. Li CY, Liu GH, Xu CC, Lee GI, Bauer P, Ling HQ, Ganal MW, Howe GA: **The tomato *Suppressor of prosystemin-mediated responses2* gene encodes a fatty acid desaturase required for the biosynthesis of jasmonic acid and the production of a systemic wound signal for defense gene expression**. *Plant Cell* 2003, **15**:1646-1661.

12. Thomma BPHJ, Eggermont K, Penninckx IAMA, Mauch-Mani B, Vogelsang R, Cammue BPA, Broekaert WF: **Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens**. *Proc Natl Acad Sci USA* 1998, **95**:15107-15111.

13. • Ton J, Van Pelt JA, Van Loon LC, Pieterse CMJ: **Differential effectiveness of salicylate-dependent and jasmonate/ethylene-dependent induced resistance in *Arabidopsis***. *Mol Plant Microbe Interact* 2002, **15**:27-34.

The spectrum of effectiveness of pathogen-induced SAR and rhizobacteria-mediated ISR is studied using pathogens that are primarily resisted in non-induced plants through either SA-dependent or JA/ET-dependent basal defense responses. SAR was mainly effective against pathogens that are inhibited by SA-dependent defense responses, whereas ISR was predominantly effective against pathogens that are sensitive to JA/ET-dependent defenses. The authors postulate that the activation of pathogen-induced SAR and rhizobacteria-mediated ISR constitute a reinforcement of extant SA- or JA/ET-dependent basal defense responses, respectively.

14. • Maldonado AM, Doerner P, Dixon RA, Lamb CJ, Cameron RK: **A putative lipid transfer protein involved in systemic resistance signalling in *Arabidopsis***. *Nature* 2002, **419**:399-403.

The authors screened a collection of T-DNA-tagged Arabidopsis lines for mutants that fail to develop SAR in response to local infection by avirulent *P. syringae*. Out of the 11 000 T-DNA lines tested, the *dir1* mutant was specifically compromised in SAR against infection by virulent *P. syringae* and *P. parasitica*. Local defense responses to virulent and avirulent *P. syringae* were unaffected in this mutant. Experiments with petiole exudates from the leaves of wildtype and *dir1* plants that were inoculated with mock- and avirulent *P. syringae* revealed that exudates from inoculated *dir1* plants are incapable of inducing *PR-1* gene expression. The authors propose that DIR1 is required for the production or transmission of a mobile signal that is required for SAR. *DIR1* encodes a putative apoplastic lipid-transfer protein, suggesting a role for lipid-derived signals in long-distance signaling.

15. • Kumar D, Klessig DF: **High-affinity salicylic acid-binding protein 2 is required for plant innate immunity and has salicylic acid-stimulated lipase activity**. *Proc Natl Acad Sci USA* 2003, **100**:16101-16106.

The authors describe the purification and characterization of tobacco SABP2, a SA-binding protein that binds SA with high affinity. SABP2 is found to be a lipase whose activity is stimulated upon SA binding. This protein is involved in the local resistance of tobacco to TMV infection, in SA-mediated *PR-1* gene expression and in SAR. The authors propose that SABP2 acts as an SA receptor that, upon binding of SA, may generate a lipid-derived mobile signal that is involved in SAR.

16. Nandi A, Welti R, Shah J: **The *Arabidopsis thaliana* dihydroxyacetone phosphate reductase gene *SUPPRESSOR OF FATTY ACID DESATURASE DEFICIENCY1* is required for glycerolipid metabolism and for the activation of systemic acquired resistance**. *Plant Cell* 2004, **16**:465-477.

17. • Verberne MC, Hoekstra J, Bol JF, Linthorst HJM: **Signaling of systemic acquired resistance in tobacco depends on ethylene perception**. *Plant J* 2003, **35**:27-32.

The authors studied the role of ET in the development of TMV-induced SAR in tobacco. Experiments involving reciprocal grafts of wildtype and ET-insensitive (Tetr) tobacco plants demonstrated that ET perception is required for the production or transmission of the long-distance SAR signal.

18. Knoester M, Pieterse CMJ, Bol JF, Van Loon LC: **Systemic resistance in *Arabidopsis* induced by rhizobacteria requires**

ethylene-dependent signaling at the site of application. *Mol Plant Microbe Interact* 1999, **12**:720-727.

19. Ryan CA: **The systemin signaling pathway: differential activation of plant defensive genes**. *Biochim Biophys Acta* 2000, **1477**:112-121.

20. Lee GI, Howe GA: **The tomato mutant *spr1* is defective in systemin perception and the production of a systemic wound signal for defense gene expression**. *Plant J* 2003, **33**:567-576.

21. • Li L, Li C, Lee GI, Howe GA: **Distinct role for jasmonate synthesis and action in the systemic wound response of tomato**. *Proc Natl Acad Sci USA* 2002, **99**:6416-6421.

The authors used reciprocal grafts of wildtype tomato plants and mutants that were affected in either JA sensitivity or JA biosynthesis to investigate the role of JA in the wound-induced signaling pathway of tomato that leads to systemic expression of *PI* genes. Wounding or *PROSYSTEMIN* overexpression induced the production of a long-distance signal that is required for the activation of *PI* gene expression in distal leaves. Locally, JA biosynthesis was essential for the production of this mobile signal. Systemically, recognition of the long-distance signal appeared to depend on JA signaling. The authors propose that JA, or a related compound from the JA biosynthetic pathway, be the transmissible wound signal.

22. Van Loon LC, Van Strien EA: **The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins**. *Physiol Mol Plant Pathol* 1999, **55**:85-97.

23. Conrath U, Pieterse CMJ, Mauch-Mani B: **Priming in plant–pathogen interactions**. *Trends Plant Sci* 2002, **7**:210-216.

24. Gaffney T, Friedrich L, Vernooij B, Negrotto D, Nye G, Uknes S, Ward E, Kessmann H, Ryals J: **Requirement of salicylic acid for the induction of systemic acquired resistance**. *Science* 1993, **261**:754-756.

25. Heck S, Grau T, Buchala A, Métraux J-P, Nawrath C: **Genetic evidence that expression of NahG modifies defence pathways independent of salicylic acid biosynthesis in the *Arabidopsis–Pseudomonas syringae* pv. *tomato* interaction**. *Plant J* 2003, **36**:342-352.

26. Van Wees SCM, Glazebrook J: **Loss of non-host resistance of *Arabidopsis* NahG to *Pseudomonas syringae* pv. *phaseolicola* is due to degradation products of salicylic acid**. *Plant J* 2003, **33**:733-742.

27. Nawrath C, Métraux J-P: **Salicylic acid induction-deficient mutants of *Arabidopsis* express PR-2 and PR-5 and accumulate high levels of camalexin after pathogen inoculation**. *Plant Cell* 1999, **11**:1393-1404.

28. Wildermuth MC, Dewdney J, Wu G, Ausubel FM: **Isochorismate synthase is required to synthesize salicylic acid for plant defence**. *Nature* 2001, **414**:562-565.

29. Nawrath C, Heck S, Parinthawong N, Métraux J-P: **EDS5, an essential component of salicylic acid-dependent signaling for disease resistance in *Arabidopsis*, is a member of the MATE transporter family**. *Plant Cell* 2002, **14**:275-286.

30. Dong X: **Genetic dissection of systemic acquired resistance**. *Curr Opin Plant Biol* 2001, **4**:309-314.

31. Kinkema M, Fan W, Dong X: **Nuclear localization of NPR1 is required for activation of *PR* gene expression**. *Plant Cell* 2000, **12**:2339-2350.

32. Després C, DeLong C, Glaze S, Liu E, Fobert PR: **The *Arabidopsis* NPR1/NIM1 protein enhances the DNA binding activity of a subgroup of the TGA family of bZIP transcription factors**. *Plant Cell* 2000, **12**:279-290.

33. Zhang Y, Fan W, Kinkema M, Li X, Dong X: **Interaction of NPR1 with basic leucine zipper protein transcription factors that bind sequences required for salicylic acid induction of the *PR-1* gene**. *Proc Natl Acad Sci USA* 1999, **96**:6523-6528.

34. Zhou JM, Trifa Y, Silva H, Pontier D, Lam E, Shah J, Klessig DF: **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**. *Mol Plant Microbe Interact* 2000, **13**:191-202.

35. Subramaniam R, Desveaux D, Spickler C, Michnick SW, Brisson N: **Direct visualization of protein interactions in plant cells**. *Nat Biotechnol* 2001, **19**:769-772.

36. •• Fan WH, Dong XN: ***In vivo* interaction between NPR1 and transcription factor TGA2 leads to salicylic acid-mediated gene activation in *Arabidopsis***. *Plant Cell* 2002, **14**:1377-1389.

Yeast two-hybrid screens for NPR1-interacting proteins revealed that members of the TGA subclass of basic leucine zipper (bZIP) transcription factors bind to NPR1 *in vitro*. The authors investigate the occurrence of NPR1–TGA interactions *in vivo*. Expression of a truncated form of TGA2 resulted in a dose-dependent abolition of TGA transcription factor function, resulting in a phenotype similar to that of the *npr1* mutant. The dominant-negative effect of the truncated TGA2 protein was abolished in the *npr1* mutant background, indicting that NPR1 and TGA2 interact *in planta*. Furthermore, a chimera reporter system was constructed to monitor TGA2 transcriptional activity *in planta* during SAR. The *in vivo* activity of TGA2 was dependent on SA and NPR1.

37. • Johnson C, Boden E, Arias J: **Salicylic acid and NPR1 induce the recruitment of trans-activating TGA factors to a defense gene promoter in *Arabidopsis***. *Plant Cell* 2003, **15**:1846-1858.

The authors investigated the role of the NPR1-interacting transcription factors TGA2 and TGA3 in the activation of *PR-1* gene expression in *Arabidopsis*. Using a chromatin immunoprecipitation assay, the authors demonstrated that both TGA2 and TGA3 bind *in vivo* to SA-responsive elements in the *PR-1* promoter in an SA- and NPR1-dependent manner.

38. • Zhang YL, Tessaro MJ, Lassner M, Li X: **Knockout analysis of *Arabidopsis* transcription factors TGA2, TGA5, and TGA6 reveals their redundant and essential roles in systemic acquired resistance**. *Plant Cell* 2003, **15**:2647-2653.

The authors constructed single, double and triple mutants in which *TGA2*, *TGA5* and *TGA6* were compromised in various combinations. They tested *PR-1* gene expression and SAR against *P. parasitica* and *P. syringae* in each of these mutants. The TGA factors were shown to play a redundant but essential role in SA-mediated activation of *PR-1* gene expression and SAR.

39. Kim HS, Delaney TP: **Over-expression of TGA5, which encodes a bZIP transcription factor that interacts with NIM1/NPR1, confers SAR-independent resistance in *Arabidopsis thaliana* to *Peronospora parasitica***. *Plant J* 2002, **32**:151-163.

40. Pontier D, Privat I, Trifa Y, Zhou JM, Klessig DF, Lam E: **Differential regulation of TGA transcription factors by post-transcriptional control**. *Plant J* 2002, **32**:641-653.

41. •• Després C, Chubak C, Rochon A, Clark R, Bethune T, Desveaux D, Fobert PR: **The *Arabidopsis* NPR1 disease resistance protein is a novel cofactor that confers redox regulation of DNA binding activity to the basic domain/leucine zipper transcription factor TGA1**. *Plant Cell* 2003, **15**:2181-2191.

TGA1 and TGA4 do not bind to NPR1 in yeast two-hybrid assays. Using an *in-planta* transient expression assay that is mechanistically similar to the yeast two-hybrid system, the authors show that TGA1 does interact with NPR1 in *Arabidopsis* leaves upon SA treatment. Yeast two-hybrid assays of chimeric TGA1 proteins in which the NPR1-interacting domain of TGA2 was swapped with the corresponding domain of TGA1, revealed a 30-amino-acid segment that is important for NPR1 interaction. TGA1 and TGA4 contain two Cys residues in this region that are missing in TGAs that interact with NPR1 *in vitro*. The authors tested the hypothesis that the formation of disulfide bonds between the Cys residues prevents TGA1 and TGA4 from interacting with NPR1 in the yeast two-hybrid system. Indeed, site-directed mutagenesis of the Cys residues transformed TGA1 and TGA4 into proteins that are capable of interacting with NPR1 in the yeast two-hybrid system. Moreover, the authors showed that *in planta*, SA-mediated changes in redox status upon the induction of SAR reduces the Cys residues of TGA1, thereby facilitating the interaction of TGA1 with NPR1 and the subsequent enhancement of SA-responsive promoter elements.

42. Cao H, Li X, Dong X: **Generation of broad-spectrum disease resistance by overexpression of an essential regulatory gene in systemic acquired resistance**. *Proc Natl Acad Sci USA* 1998, **95**:6531-6536.

43. Friedrich L, Lawton K, Dietrich R, Willits M, Cade R, Ryals J: **NIM1 overexpression in *Arabidopsis* potentiates plant disease resistance and results in enhanced effectiveness of fungicides**. *Mol Plant Microbe Interact* 2001, **14**:1114-1124.

44. •• Mou Z, Fan WH, Dong XN: **Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes**. *Cell* 2003, **113**:935-944.

The accumulation of SA in response to pathogen infection creates a shift toward more reducing conditions in the plant cell. By measuring changes in the glutathione pool, which are indicative of redox changes in plant cells, the authors show that the induction of SAR is indeed associated with a change in redox homeostasis. The immunodetection of NPR1 in protein extracts from control and 2,6-dichloroisonicotinic acid (INA)-induced *Arabidopsis* leaves revealed that, in non-induced plants, NPR1 is present in an inactive oligomeric complex that is formed through intermolecular disulfide bonds. The induction of SAR resulted in the reduction of NPR1 to an active monomeric form. Mutation of two Cys residues that are crucial for the formation of NPR1 oligomers led to constitutive monomerization and nuclear localization of NPR1, resulting in constitutive *PR-1* gene expression. The cellular redox changes induced as a result of SA accumulation connect the SA signal with NPR1 activity during SAR.

45. Pieterse CMJ, Ton J, Van Loon LC: **Cross-talk between plant defence signalling pathways: boost or burden?** *AgBiotechNet* 2001, **3**:ABN 068.

46. •• Spoel SH, Koornneef A, Claessens SMC, Korzelius JP, Van Pelt JA, Mueller MJ, Buchala AJ, Métraux J-P, Brown R, Kazan K *et al.*: **NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol**. *Plant Cell* 2003, **15**:760-770.

The authors investigate the role of NPR1 in the antagonistic effect of SA on JA signaling. SA-deficient *Arabidopsis* plants produced significantly higher levels of JA, and showed enhanced JA-responsive gene expression after infection by *P. syringae* pv. *tomato*. This indicates that pathogen-induced SA accumulation is associated with the suppression of JA signaling in wildtype plants. Pharmacological experiments with *npr1* plants revealed that the antagonistic effect of SA on JA signaling is regulated through NPR1. Using a dexamethasone (DEX)-inducible system to control the nucleocytoplasmic localization of a NPR1 fusion protein, it was shown that nuclear localization of NPR1 is not required for the suppression of JA-responsive gene expression. The results presented indicate that cytosolic NPR1 plays a crucial role in cross-communication between SA- and JA-dependent plant defense responses.

47. Devoto A, Nieto-Rostro M, Xie DX, Ellis C, Harmston R, Patrick E, Davis J, Sherratt L, Coleman M, Turner JG: **COI1 links jasmonate signalling and fertility to the SCF ubiquitin-ligase complex in *Arabidopsis***. *Plant J* 2002, **32**:457-466.

48. Xu L, Liu F, Lechner E, Genschik P, Crosby WL, Ma H, Peng W, Huang D, Xie D: **The SCF^{COI1} ubiquitin-ligase complexes are required for jasmonate response in *Arabidopsis***. *Plant Cell* 2002, **14**:1919-1935.

49. • Li J, Brader G, Palva ET: **The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense**. *Plant Cell* 2004, **16**:319-331.

The authors identified the *Arabidopsis* transcription factor WRKY70 as a key factor in controlling plant defense responses to infection by *Erwinia carotovora*. The expression of the *WRKY70* gene was induced by SA and elicitors from *E. carotovora*, but was repressed by JA. Overexpression of *WRKY70* constitutively activated the SA-responsive *PR-1* gene and suppressed several JA-responsive genes. NPR1 was found to play a regulatory role in both processes. The authors propose that the balance between SA and JA levels attained after pathogen infection determines the level of *WRKY70* expression. High levels of WRKY70 lead to SA-responsive gene expression, whereas low levels of WRKY70 favor the activation of JA-related genes.

50. • Lorenzo O, Piqueras R, Sánchez-Serrano JJ, Solano R: **ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense**. *Plant Cell* 2003, **15**:165-178.

ERF1 is a transcription factor that acts downstream of the ET signaling pathway. It has been implicated in the regulation pathogen-responsive genes and disease resistance in *Arabidopsis*. The authors show that *ERF1* is rapidly activated in response to both ET and JA, and that a combination of both of these hormones leads to enhanced expression of the *ERF1* gene. Analysis of the *ethylene insensitive2* (*ein2*) mutant and the JA-response mutant *coronatine insensitive1* (*coi1*) revealed the ET and JA are simultaneously required for *ERF1* gene activation. Overexpression of *ERF1* in the *coi1* background resulted in the constitutive expression of a set of JA-responsive genes whose activation was blocked in this mutant. Previously, similar observations were made in *ERF1*-overexpressing *ein2* plants, indicating that ERF1 acts downstream of both ET and JA in activating the expression of genes that are co-regulated by these hormones. The authors propose that ERF1 is a key regulatory factor in integrating both hormone signals to control defense-related gene expression.

51. Chen Z, Silva H, Klessig DF: **Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid**. *Science* 1993, **262**:1883-1886.

52. Mittler R: **Oxidative stress, antioxidants and stress tolerance**. *Trends Plant Sci* 2002, **7**:405-410.

53. Shah J: **The salicylic acid loop in plant defense**. *Curr Opin Plant Biol* 2003, **6**:365-371.

54. Alvarez ME, Pennell RI, Meijer PJ, Ishikawa A, Dixon RA, Lamb C: **Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity**. *Cell* 1998, **92**:773-784.

55. Desveaux D, Subramaniam R, Després C, Mess J-N, Lévesque C, Fobert PR, Dangl JL, Brisson N: **A 'Whirly' transcription factor is required for salicylic acid-dependent disease resistance in *Arabidopsis***. *Dev Cell* 2004, **6**:229-240.