New mutants provide clues into regulation of systemic acquired resistance

When plants encounter pathogens, resistance mechanisms are activated that can prevent infection, aid recovery from disease and prevent future infection. An important component in a plant's defense arsenal is the pathogen-induced response called systemic acquired resistance (SAR), which when activated can prevent infection by a wide range of pathogens. SAR was described in 1961 by Frank Ross (Cornell University, USA) and later found by others to be associated with the induction of a suite of pathogenesis-related (PR) genes and their corresponding proteins. Salicylic acid is an endogenous signaling molecule, which is required for the induction of SAR. Application of salicylic acid or its synthetic analogs [2,6-dichloroisonicotinic acid (INA) or benzo (1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester (BTH)] to plants induces PR gene expression and resistance as would a biological agent. In addition, transgenic plants that express salicylate hydroxylase, which is encoded by the bacterial *nahG* gene, can neither accumulate salicylic acid after pathogen attack, nor activate SAR (reviewed in Refs 1,2). In the past several years, genetic analysis has revealed components in the pathway that regulates SAR by identifying mutants perturbed in this response. The recent description of a suppressor mutation that restores function to SAR mutants, and the cloning of its gene, has generated new insights into how this important plant defense response is regulated[3].

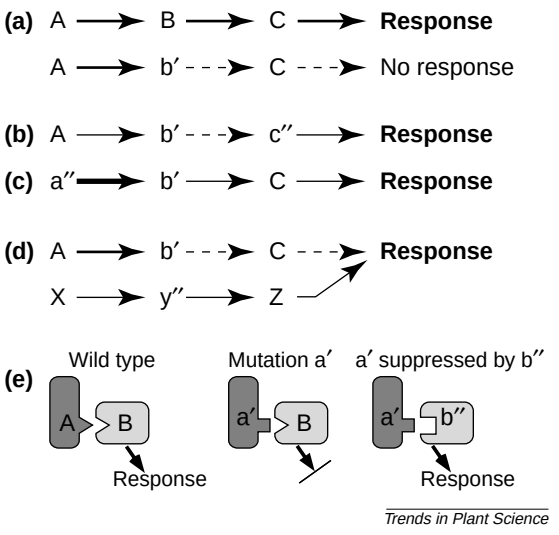

Fig. 1. Genetic suppressor classes. (a) Generic signal transduction pathway leading to a response; mutation of B function (b′) disrupts the pathway, preventing a response. (b–e) Second site suppressor mutations (indicated by a double prime) can suppress the phenotype caused by primary mutation b′, restoring the response. Suppressor mutations can act downstream (b), upstream (c), or in pathways separate from that implicated by the primary mutation (d). (e) Certain suppressors can restore function to an inactive product of a primary mutation by restoring functional interaction between both proteins.

An *Arabidopsis* gene required for SAR activation

In the early 1990s, *Arabidopsis thaliana* was shown to be a useful genetic model for studying SAR, enabling several independent groups to initiate screens to identify SAR-defective mutants. SAR-deficient mutants were obtained in John Ryals' laboratory by treating mutagenized plants with INA, followed by challenge with downy mildew (*Peronospora parasitica*); plants showing downy mildew disease were found to be defective in SAR and called *nim1* mutants (*non-inducible immunity*)[4]. Taking a different approach, Xinnian Dong's group screened transgenic plants containing the *BGL2* (PR-2) promoter driving a *uidA* (GUS) reporter gene. They found mutants that failed to show induction of the reporter after INA treatment, which they called *npr1* mutants (*non-inducer of PR genes*)[5]. Both *nim1* and *npr1* mutants exhibit the same salicylic acid-insensitive phenotype, and later in allelism tests were found to have mutations in the same gene, hereafter called *NIM1*/*NPR1*. Subsequent work in other laboratories using different mutant screens identified additional mutant alleles at this locus[6,7].

The *NIM1*/*NPR1* gene was cloned independently in two laboratories and found to encode a novel protein that contains multiple ankyrin repeats, motifs known to mediate interaction with other proteins[8,9]. It was further proposed that the protein is a possible homolog of Iκ-B and Cactus regulatory proteins[9], found in vertebrates and flies respectively, which regulate the activity of the Rel-family transcription factors NF-κB and Dorsal. These signal transduction pathways (STPs) have been studied thoroughly and most of the pathway components have been identified. Both the Iκ-B and Cactus pathways are composed of structurally and functionally homologous components that link perception of extracellular signals to changes in gene expression. The Iκ-B and Cactus pathways provide control of the innate immune system, a general defense response that is activated by the perception of a pathogen, and initiates the production of potent antimicrobial peptides and other defense molecules[10]. SAR also functions as an innate immune system in plants because it is pathogen-induced and leads to the production of a range of anti-microbial compounds.

The discovery that *Arabidopsis* NIM1/NPR1 is a possible Iκ-B and Cactus homolog is intriguing because many plant disease-resistance genes appear to have homology with other components in the Iκ-B and Cactus pathways in animals (reviewed in Refs 11,12). If animal and plant defense pathways are structurally and functionally homologous, they must have an ancient origin that pre-dates the divergence of plants and animals.

Identification of NIM1/NPR1 partner proteins

To find other components of the SAR STP, several groups have taken advantage of the yeast two-hybrid system to screen for plant genes whose products interact directly with NIM1/NPR1. Xinnian Dong's group recently described their success in finding *Arabidopsis* bZIP family transcription factors that interact with NIM1/NPR1 (Ref. 13). Other research groups have obtained similar results, although the specific bZIP proteins implicated differ somewhat (D. Klessig, pers. commun.; H. Kim and T. Delaney, unpublished). These findings suggest that NIM1/NPR1 and bZIP transcription factors are involved coordinately in the regulation of SAR. However, to date, no evidence has been shown that demonstrates *in planta* interaction of NIM1/NPR1 with bZIP factors, or that alteration of the bZIP activity has an affect on induced resistance. These observations will be important for assessing the role proposed for bZIP proteins in regulating SAR.

Although SAR-nonresponsive mutants have been sought in several laboratories using a variety of mutant screens, only one gene (*NIM1*/*NPR1*) has been found to be required for salicylic acid-mediated induction of resistance. Failure to identify other genes in

loss-of-function genetic screens might indicate that the STP that links the salicylic acid signal to the induction of PR genes and resistance is short. Alternatively it might indicate that other pathway components exist but are redundant or essential for plant viability, making them difficult to identify by mutation. Thus, to further dissect the SAR pathway by mutation analysis, other approaches were needed that were not limited by these complications.

Application of suppressor mutant analysis

A powerful method to discover additional components of an STP is to screen for second site mutations that suppress a phenotype caused by a primary mutation[14] (Fig. 1a). Such suppressor mutations might implicate gene products that act downstream of the primary lesion in the STP (Fig. 1b). Alternatively, hyperactive alleles of genes whose products act upstream in the STP can suppress partially functional primary mutations (Fig. 1c). However, some suppressor mutations might restore function via mechanisms independent of the pathway implicated by a primary mutation (Fig. 1d). If the primary mutation does not eliminate the gene's product, then compensatory mutations in a gene encoding an interacting protein can restore function and thus produce a suppressed phenotype (Fig. 1e). Such compensatory mutations are likely to be highly allele-specific, by functioning with only specific mutant alleles of the primary mutation. Allele-specific suppressors are particularly interesting because they might implicate proteins that interact physically with a known STP component.

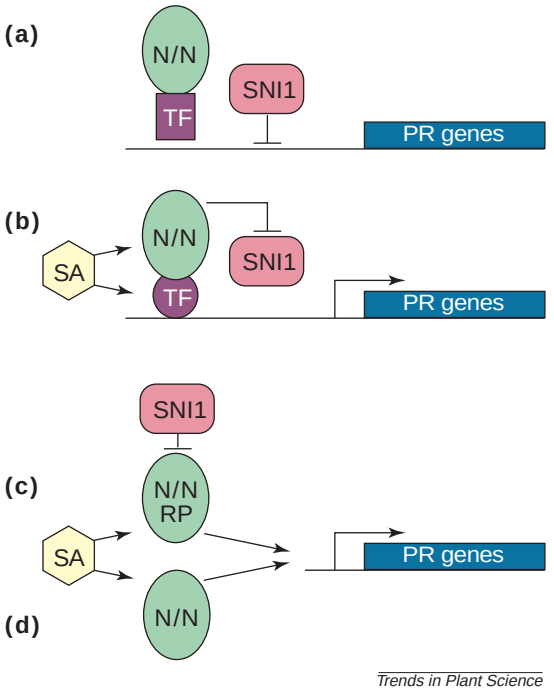

Fig. 2. Models for regulation of pathogenesis-related (PR) genes. (a) In the absence of salicylic acid (SA), PR gene expression is suppressed by the action of the SNI1 repressor. (b) Salicylic acid activates NIM1/NPR1 (N/N), which represses the action of the SNI1 repressor; PR genes are then expressed. SA also activates a second factor, possibly a transcription factor (TF) that can activate PR gene expression in the absence of both N/N and SNI1, such as is found in *sni1 npr1-1* double mutant suppressed plants. (a) and (b) are adapted, with permission, from Ref. 3. (c–d) Phantom pathway model. Wild-type plants employ N/N to transmit salicylic acid signals, leading to the activation of PR gene expression. Another salicylic acid-responsive pathway exists, but is masked in certain tissues or conditions by the action of the SNI1 repressor or other SNI1-dependent factors. The phantom salicylic acid-responsive element might be a NIM1/NPR1-related protein (N/N RP) found in *Arabidopsis*. Mutations that remove N/N activity block the salicylic acid induction of PR genes, whereas the removal of both N/N and the SNI1 repressor exposes the N/N RP, which then transmits the salicylic acid signal.

To dissect the *Arabidopsis* SAR pathway further, several laboratories have employed genetic-suppressor screens, screening mutagenized *nim1*/*npr1* populations for plants that show partial or full recovery of the SAR phenotype. The semi-dominant *ssi1-1* mutation (*suppressor of salicylic acid-insensitivity*), which partially suppresses the *npr1-5* mutant phenotype, has been described recently[15]. Homozygous or heterozygous *ssi1* plants exhibit constitutive accumulation of several PR mRNAs, contain high levels of salicylic acid, are dwarfed and have lesions. The Ssi1$^-$ (suppressed) phenotype is lost when combined with NahG, showing its dependence upon salicylic acid accumulation. Suppression by *ssi1-1* is not allele-specific, because both *npr1-5* and *nim1-3* (probably a null allele) plants exhibit the same constitutive defense gene-expression phenotype when combined with *ssi1*. This led to the conclusion that SSI1 defines an STP that is distinct from the NIM1/NPR1 pathway, yet is dependent upon salicylic acid accumulation. Thus, the *ssi1* suppressor appears not to identify additional components of the NIM1/NPR1 pathway, but rather another pathway that can mediate PR gene expression independent of NIM1/NPR1.

A recent report[3] described a suppressor mutation corresponding to the *SNI1* gene (*suppressor of npr1 inducible*), which was identified in a screen for suppressors that restore INA-induced BGL2 expression in *npr1-1* mutant plants. Plants homozygous for both *npr1-1* and the recessive *sni1-1* allele show a nearly wild-type phenotype, remarkably regaining INA and salicylic acid induction of PR gene expression and resistance to pathogens. The *sni1 npr1-1* double mutant plants are also similar to wild-type plants with respect to low endogenous salicylic acid levels and normal accumulation of salicylic acid after pathogen infection, although *sni1* plants are dwarfed relative to wild type. Suppression of *npr1-1* by the *sni1* mutation requires salicylic acid, because NahG plants harboring both mutations do not show induction of PR genes by INA. Together, these observations indicate that SNI1 acts downstream of salicylic acid, as does NIM1/NPR1. SNI1 is not believed to interact physically with NPR1. This is based on negative results from yeast two-hybrid analysis and because the *sni1-1* mutation also suppresses a wide range of other *npr1* mutant alleles (*npr1-1*, *npr1-2*, *npr1-3* and *npr1-4*; X. Dong, pers. commun.). *SNI1* has been cloned: it encodes a novel 48 kd protein, which in the *sni1* mutant is presumably absent or truncated because of the nature of the mutation[3].

Models for SAR pathway regulation

Suppression of the *npr1-1* mutant phenotype by *sni1* suggests that either the NIM1/NPR1 pathway is somehow restored to normal function in suppressed (*sni1 npr1-1* double mutant) plants, or that an alternate salicylic acid-response pathway is active in suppressed plants. Restoration of the normal function of the NIM1/NPR1 pathway is elucidated in a model[3] (Fig. 2): it is proposed that SNI1 acts as a repressor of PR gene expression. In this model, salicylic acid activates NIM1/NPR1, which then represses the SNI1 repressor, allowing transcription of PR genes. The model also invokes a second salicylic acid-activation that affects another regulatory factor, possibly one of the bZIP transcription factors found in the two-hybrid screen to interact with NIM1/NPR1. The second salicylic acid-activation is postulated to explain the salicylic acid-inducible responses found in *sni1 npr1-1* double mutants.

An alternative model to that described above postulates the existence of a redundant salicylic acid-response pathway that can mediate responses to salicylic acid in *sni1* mutants[3]. In this model, the phantom salicylic acid-response pathway is masked in wild-type and *nim1*/*npr1* mutants by the action of a repressor, possibly SNI1 itself or another factor dependent upon SNI1 (Fig. 2). However, in an

sni1 mutant, the phantom pathway is exposed and salicylic acid responses are regained. Because *Arabidopsis* has several genes closely related to *NIM1*/*NPR1* (see *Arabidopsis* Genome Research Project databases; T. Delaney, unpublished), it is possible that the proposed phantom pathway employs one of the NIM1/NPR1 homologs to respond to salicylic acid signals, but shares upstream and downstream pathway components with the authentic NIM1/NPR1 STP. The phantom pathway model is economical, because just one mode of salicylic acid response is required, provided that other members of the gene family share with NIM1/NPR1 the ability to transmit salicylic acid-derived signals. If an alternative salicylic acid-response pathway does exist, one must wonder under what conditions the phantom pathway is active, and in what tissues or under what conditions SNI1 activity is found. A clue might come from the pattern of INA-induced GUS expression in BGL2-*uidA* transgenic plants. In an *sni1 npr1-1* double mutant background, GUS expression is evident around the vascular tissues of leaves and roots, suggesting that SNI1 represses expression of PR genes in those tissues[3]. Repression of PR gene expression by SNI1 might also be important for plant fitness, because *sni1* mutants have a dwarfed phenotype.

Other observations have suggested the existence of an NIM1/NPR1-independent salicylic acid-response pathway. These include the persistent, yet reduced expression of PR genes in pathogen-challenged *nim1*/*npr1* plants, suggesting that NIM1/NPR1 is just one way that pathogen perception is linked to PR gene expression[4,6]. Furthermore, the dominant *ssi1* mutation causes constitutive PR-1 gene expression in a salicylic acid-dependent, yet NIM1/NPR1-independent manner. This suggests a role for SSI1 in an alternate pathway that controls expression of defense genes[15]. It will be interesting to learn whether *ssi1* and *sni1* mutations implicate the same signaling pathway. Finally, genes have been identified that require salicylic acid for induction, independent of NIM1/NPR1 (G. Rairdan, N. Donofrio and T. Delaney, unpublished).

Future prospects

Additional work will show whether SNI1 acts within the NIM1/NPR1 pathway, or identifies another that responds to salicylic acid. A variety of other suppressor mutants are being actively pursued in several laboratories (X. Dong, pers. commun.; D. Klessig, pers. commun.; T. Delaney, unpublished). Some of these might identify genes whose products interact directly with NIM1/NPR1. For example, two of the mutants we are examining show specific suppression of *nim1-1* but not *nim1-2* or *nim1-5* plants, suggesting that they might implicate proteins acting within the NIM1/NPR1 pathway (H. Kim and T. Delaney, unpublished). The strategy of screening for mutants in plants harboring primary mutations has proven effective, and promises to help unravel the complex network of pathways that control pathogen-induced disease resistance. Better understanding is needed of this important part of plant biology, and will also enable rational approaches for enhancing natural disease resistance in plants.

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References

1 Ryals, J. *et al.* (1996) Systemic acquired resistance. *Plant Cell* 8, 1809–1819
2 Delaney, T.P. (1997) Genetic dissection of acquired resistance to disease. *Plant Physiol.* 106, 5–12
3 Li, X. *et al.* (1999) Identification and cloning of a negative regulator of systemic acquired resistance, SNI1, through a screen for suppressors of *npr1-1*. *Cell* 98, 329–339
4 Delaney, T.P. *et al.* (1995) *Arabidopsis* signal transduction mutant defective in chemically and biologically induced disease resistance. *Proc. Natl. Acad. Sci. U. S. A.* 92, 6602–6606
5 Cao, H. *et al.* (1994) Characterization of an *Arabidopsis* mutant that is nonresponsive to inducers of systemic acquired resistance. *Plant Cell* 6, 1583–1592
6 Glazebrook, J. *et al.* (1996) Isolation of *Arabidopsis* mutants with enhanced disease susceptibility by direct screening. *Genetics* 143, 973–982
7 Shah, J. *et al.* (1997) Characterization of a salicylic acid-insensitive mutant (*sai1*) of *Arabidopsis thaliana*, identified in a selective screen utilizing the SA-inducible expression of the *tms2* gene, *Mol. Plant–Microbe Interact.* 10, 69–78
8 Cao, H. *et al.* (1997) The *Arabidopsis NPR1* gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. *Cell* 88, 57–63
9 Ryals, J. *et al.* (1997) The *Arabidopsis* NIM1 protein shows homology to the mammalian transcription factor inhibitor IκB. *Plant Cell* 9, 425–439
10 Hoffmann, J.A. *et al.* (1999) Phylogenetic perspectives in innate immunity. *Science* 284, 1313–1318
11 Glazebrook, J. *et al.* (1997) Use of *Arabidopsis* for genetic dissection of plant defense responses. *Annu. Rev. Genet.* 31, 547–569
12 Wilson, I. *et al.* (1997) Signalling pathways: a common theme in plants and animals? *Curr. Biol.* 7, 175–178
13 Zhang, Y. *et al.* (1999) 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. U. S. A.* 96, 6523–6528
14 Prelich, G. (1999) Suppression mechanisms: themes from variations. *Trends Genet.* 15, 261–266
15 Shah, J. *et al.* (1999) The *Arabidopsis ssi1* mutation restores pathogenesis-related gene expression in *npr1* plants and renders defensin gene expression salicylic acid dependent. *Plant Cell* 11, 191–206

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