The salicylic acid loop in plant defense

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Salicylic acid is an important signal molecule in plant defense. In the past two years, significant progress has been made in understanding the mechanism of salicylic-acid biosynthesis and signaling in plants. A pathway similar to that found in some bacteria synthesizes salicylic acid from chorismate via isochorismate. Salicylic-acid signaling is mediated by at least two mechanisms, one requiring the *NON-EXPRESSOR OF PR1* (*NPR1*) gene and a second that is independent of *NPR1*. Feedback loops involving salicylic acid modulate upstream signals. These feedback loops may provide a point for integrating developmental, environmental and other defense-associated signals, and thus fine-tune the defense responses of plants.

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Abbreviations

BTH	benzo (1,2,3) thiadiazole-7-carbothioic acid *S*-methyl ester
coi1	*coronatine insensitive1*
cpr5	*constitutive expressor of PR genes5*
α-DOX1	α-dioxygenase1
eds16	*enhanced disease susceptibility16*
ein2	*ethylene insensitive2*
GAL4	GALACTOSE UTILIZATION4
GUS	β-glucuronidase
HR	hypersensitive response
ICS	isochorismate synthase
IPL	isochorismate pyruvate lyase
JA	jasmonic acid
jar1	*JA-insensitive1*
LRR	leucine-rich repeat
NahG	salicylate hydroxylase gene
NBS	nucleotide-binding site
NPR1	*NON-EXPRESSOR OF PR1*
pad4	*phytoalexin deficient4*
PR	pathogenesis-related
R	resistance gene
SA	salicylic acid
SAR	systemic acquired resistance
sfd1	*suppressor of fatty-acid-desaturase deficiency1*
SID2	*SALICYLIC-ACID-INDUCTION DEFICIENT2*
ssi2	*suppressor of SA-insensitivity2*
TGA	TGA-element binding protein
TIR	toll-interleukin-2 receptor
UAS	upstream activation sequence

Introduction

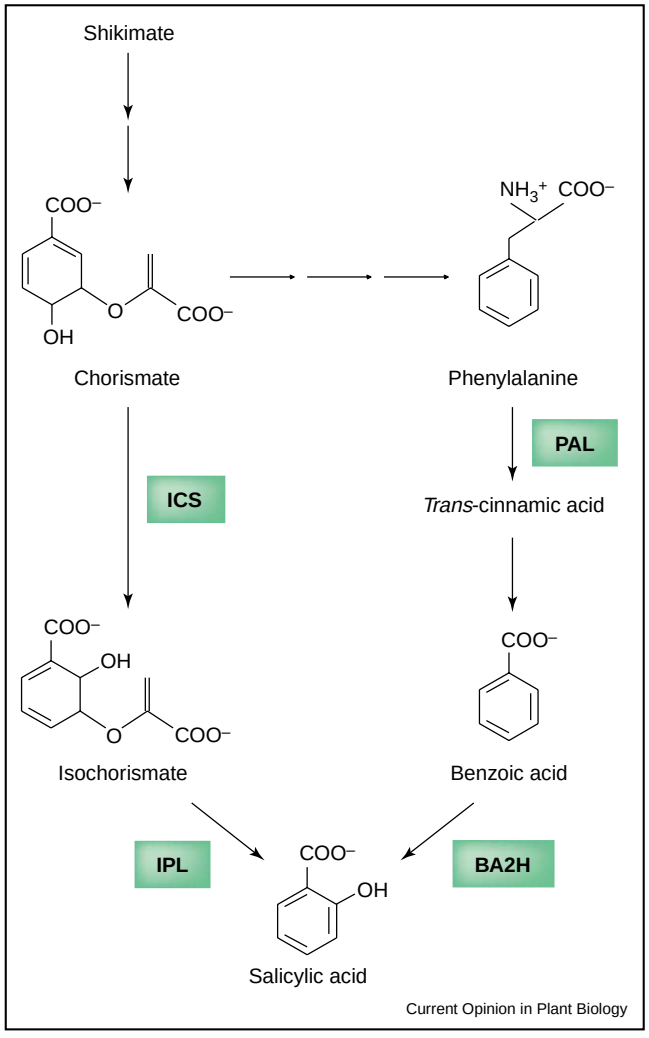
The involvement of salicylic acid (SA) as a signal molecule in local defenses and in systemic acquired resistance (SAR) has been extensively studied [1,2]. SA is also required for symptom development [3]. Increases in the endogenous levels of SA and its conjugates in pathogen-inoculated plants coincide with the elevated expression of genes encoding the pathogenesis-related (PR) proteins and the activation of disease resistance. Preventing SA accumulation, by degrading it to catechol in transgenic plants that express the bacterial salicylate hydroxylase gene (*NahG*) [4,5] or by blocking SA synthesis [6••,7], effectively blocks the activation of SA-dependent defense responses. By contrast, the exogenous application of SA or its synthetic functional analog benzo (1,2,3) thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) results in the activation of *PR*-gene expression and enhanced resistance to pathogens. The *Arabidopsis thaliana NON-EXPRESSOR OF PR1* (*NPR1*) gene, also called *NON-INDUCIBLE IMMUNITY1* (*NIM1*), is an important component of SA signaling. *npr1* and *nim1* mutant plants are insensitive to SA, and this compromises their disease resistance [8–11]. An NPR1-independent, SA-mediated resistance mechanism(s) also operates in *Arabidopsis* [12].

Genetic screens in *Arabidopsis* have identified genes that are involved in SA synthesis and signaling. This review focuses on recent studies on SA synthesis and signaling, emphasizing recent research carried out in *Arabidopsis*. In addition, I summarize evidence that supports the involvement of a SA-feedback loop in modulating upstream signals. Cross-talk between SA and other signaling pathways, which is important for the fine tuning of plant defense, has been extensively covered in recent reviews [13–15] and hence is not reviewed here.

SA biosynthesis

Previous studies suggested that SA is synthesized from phenylalanine (Figure 1; reviewed in [16]). However, this pathway cannot account for all of the SA in plant cells, suggesting the presence of an alternative biosynthesis pathway for SA. In some bacteria, SA is synthesized from chorismate via isochorismate. The enzymes isochorismate synthase (ICS) and isochorismate pyruvate lyase (IPL) catalyze the two steps from chorismate to SA [17]. Recent studies have shown that overexpression of these two bacterial enzymes in transgenic plants enhances SA accumulation [18•,19]. A study in *Arabidopsis* supports the existence of an analogous pathway in plants [6••]. Expression of the *Arabidopsis SALICYLIC-ACID-INDUCTION DEFICIENT2* (*SID2*) gene, which encodes a putative chloroplast-localized ICS, is activated in tissues that are

Figure 1



Proposed pathways for the biosynthesis of SA in plants. The shikimate pathway provides chorismate, which can be converted into SA. The main chorismate pool occurs in the chloroplast. The *Arabidopsis SID2* gene encodes a predicted ICS that has a putative plastid-transit sequence. The *SID2*-encoded ICS is proposed to catalyze the conversion of chorismate to isochorismate, presumably in the chloroplast. By analogy to the mechanism of SA biosynthesis in bacteria, it is suggested that an IPL catalyzes the conversion of isochorismate to SA. An alternative pathway that has been studied in tobacco synthesizes SA from phenylalanine via benzoic acid. Phenylalanine ammonia lyase (PAL) catalyzes the first step in this pathway, which is the conversion of phenylalanine to *trans*-cinnamic acid. *Trans*-cinnamic acid is subsequently converted into benzoic acid. A benzoic-acid-2-hydroxylase (BA2H) catalyzes the final step, the conversion of benzoic acid to SA.

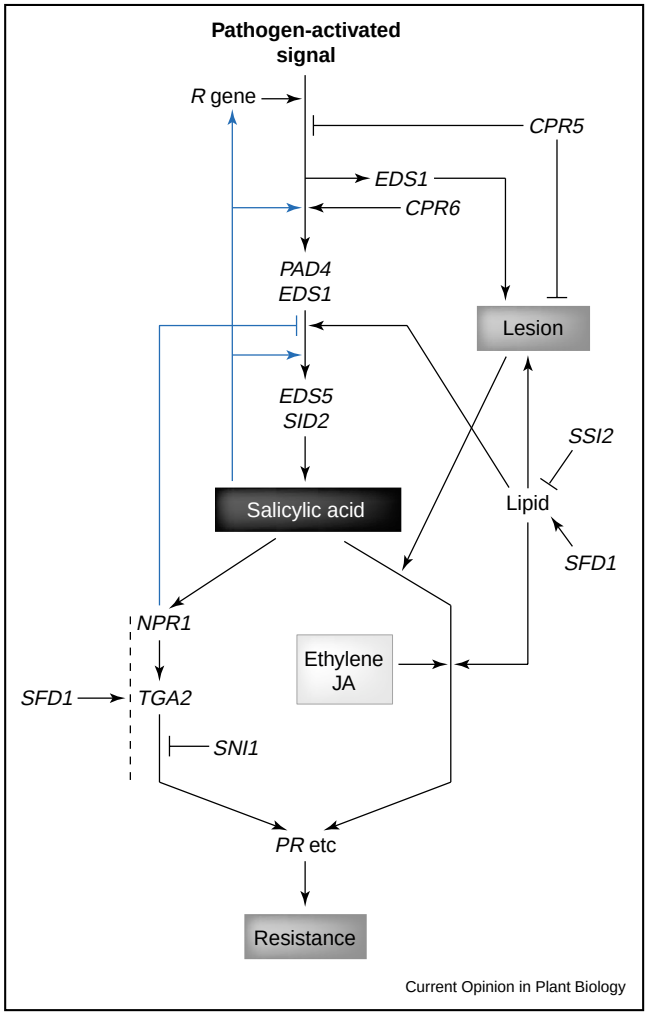
challenged by pathogens and in tissues exhibiting SAR. Moreover, the *sid2* and the allelic *enhanced disease susceptibility16* (*eds16*) mutants are defective in SA synthesis and SAR activation, and exhibit enhanced susceptibility to pathogens [6••,7]. Application of SA complements the *sid2* defect, confirming the involvement of SID2 in SA synthesis. Although the biochemical activity of SID2 has not been tested, available evidence strongly supports the existence of an alternative SA-synthesis pathway that involves chorismate and isochorismate in *Arabidopsis*.

The *EDS5* gene encodes a predicted protein that has homology to the multidrug and toxin extrusion (MATE) family of transporter proteins [20]. The activation of SA synthesis by exposure to pathogens or ultraviolet light is blocked in the *eds5*/*sid1* mutant. EDS5 may have evolved to transport phenolic compounds that are precursors of SA biosynthesis [20]. The *eds1* and *phytoalexin deficient4* (*pad4*) mutant alleles compromise SA synthesis in plant–pathogen interactions [21•,22] and in constitutive SA-signaling mutants [23,24]. *eds1* and *pad4* also block the pathogen-activated expression of *EDS5*, suggesting that EDS1 and PAD4 function upstream of EDS5 in regulating SA synthesis (Figure 2; [20]). Several *Arabidopsis* mutants that constitutively accumulate high levels of SA, express *PR* genes and exhibit enhanced resistance have also been identified [12–14]. Although not all of these genes may be associated directly with plant defense, some may link upstream events in the plant–pathogen interaction with SA synthesis, whereas others may target feedback mechanisms that involve SA, and thus fine-tune SA signaling.

NPR1-dependent SA signaling

npr1 mutant alleles were first identified in screens for *Arabidopsis* mutants that were unable to activate the expression of *PR* genes or disease resistance in response to SAR-activating conditions [8–11]. Overexpression of NPR1 enhances resistance in *Arabidopsis* and rice [25–27]. Application of SA or its analogs stimulates the translocation of NPR1 into the nucleus [28], which is required for the activation of downstream signaling. NPR1 contains an ankyrin-repeat domain, which in other proteins mediates protein–protein interaction. Some of the *npr1* mutants contain mutations in this domain, highlighting its importance to NPR1 function. Members of the TGA-element binding protein (TGA) family of basic-leucine-zipper (bZIP) DNA-binding proteins interact physically with NPR1 in yeast two-hybrid assays [12]. TGAs were originally identified as proteins that bind the DNA element known as *as-1*, which is present in the promoters of various plant and viral genes [29]. Fan and Dong [30••] performed a set of elegant experiments to show that the *Arabidopsis* TGA2 is involved in NPR1-mediated SA signaling. They showed that overexpression of a truncated form of TGA2 (TGA2CT), which lacks the DNA-binding domain, has a dominant-negative effect and confers a phenotype similar to that of the *npr1* mutant. They further demonstrated that NPR1 interacts with the TGA2CT protein, and that SA enhances this interaction. Moreover, a chimeric protein, containing the GALACTOSE UTILIZATION4 (GAL4) DNA-binding domain and the TGA2 transactivation domain, TGA2–GAL4,

Figure 2



Model for the SA signaling network in *Arabidopsis thaliana*. A pathogen-activated signal stimulates the production of SA in plants. Activation of *R*-gene-mediated signaling also activates SA synthesis. In addition, it stimulates the development of the lesions associated with the HR. *EDS1* is required at two stages. It is required for the development of a HR and for the activation of defense signaling mediated by the TIR–NBS–LRR type of *R* genes. In addition, *EDS1* and *PAD4* are required for basal resistance and for increased SA accumulation in response to challenge by various pathogens. *EDS5* most likely acts upstream of *SID2* in regulating SA biosynthesis, as the pathogen-induced expression of *EDS5* is unaltered in the *sid2* mutant. SA can activate expression of the pathogenesis-related (*PR*) gene and resistance via two mechanisms. The first, requires the *NPR1* gene. Interaction of NPR1 with TGA2 activates the expression of the *PR-1* gene, presumably by countering the inhibitory effect of SUPPRESSOR OF npr1-1 INDUCIBLE1 (SNI1). An SFD1-generated lipid signal is required for the activation of the NPR1 pathway by SA. The exact point of entry of this SFD1-derived signal in the NPR1 pathway is not known (indicated by a broken line). SA and BTH do not activate resistance in the *npr1* mutant, and so the NPR1-independent, SA-dependent mechanism requires a second signal in addition to SA. This second signal could be provided by cell death. Ethylene and JA signaling potentiate signaling through this NPR1-independent pathway. The *cpr5*, *cpr6* and *ssi2* mutants activate signaling through NPR1-dependent and -independent pathways. The *eds1*, *pad4* and *eds5* alleles compromise the dominant *cpr6*-conferred resistance. Hence, *cpr6* is shown to act before the requirement for *EDS1* plus *PAD4* for SA synthesis. The *cpr5* mutant spontaneously develops lesions, which are only partially suppressed by the *eds1* and *pad4* mutations. Hence, *CPR5* is shown to repress lesions independently of *EDS1* and *PAD4*. The wildtype *SSI2* gene represses plant defense by affecting the generation of a lipid molecule that is required for the activation of SA synthesis and the NPR1-independent pathway. Since, *eds5* but not *pad4* suppresses *ssi2*-conferred SA accumulation, this lipid signal is shown to act after *PAD4* but before *EDS5*, leading to SA synthesis. *SSI2* also represses lesion development. The wildtype *SFD1* is required for the generation of a lipid signal that is required for the expression of *ssi2*-conferred phenotypes. The wildtype *NPR1* allele represses SA accumulation through a feedback mechanism. Pathogen-activated expression of *SID2* was higher in the *npr1* mutant, and so this negative feedback regulation is exerted before SID2 action. In addition, a positive feedback mechanism involving SA enhances the expression of several *R* genes, and of *EDS1*, *PAD4*, *EDS5* and *SID2*. Arrows indicate positive effects whereas lines ending with a bar indicate inhibitory effects. Blue lines indicate feedback regulation.

activated a UAS^{GAL}::β-glucuronidase (GUS) reporter construct in response to SA. NPR1 was required for the SA-activated expression of this reporter A mutant derivative of the tobacco protein TGA2.2, which lacks DNA-binding activity, also suppressed SA-activated *PR-1* expression in transgenic tobacco plants [31]. In striking contrast, however, overexpression of a dominant-negative *Arabidopsis* TGA2, which lacks DNA-binding activity and suppresses all *as-1*-element-binding activity in transgenic tobacco plants, caused hypersensitivity to SA. Unlike wildtype plants, these transgenic tobacco plants exhibited heightened induction of *PR* genes by SA application and pathogen challenge [32]. Disparate interactions between the heterologous *Arabidopsis* TGA2 and tobacco proteins could account for the phenotypes in these transgenic tobacco plants. Alternatively, the above studies suggest that TGA factors can function as both positive and negative regulators of SA-dependent gene expression. Indeed, positive and negative regulatory elements are found in the promoters of the *Arabidopsis PR1* gene [33]. The biological significance of NPR1-interaction with TGA factors other than TGA2 is unclear. However, overexpression of TGA5, which interacts with NPR1 in yeast two-hybrid screens, confers SA- and NPR1-independent resistance to *Peronospora parasitica* in *Arabidopsis* [34]. This evidence suggests that TGA5 at least might be involved in other plant defense mechanisms.

SA application and pathogen-inoculation enhance *NPR1* expression. Although we do not know the significance of this increase in *NPR1* expression for the activation of NPR1-dependent defense responses, we do know that the binding of an SA-inducible protein complex to a W-box in the promoter of *NPR1* is necessary for the expression of *NPR1* [35•]. The WRKY family of proteins binds W-boxes. A mutant W-box element in the promoter of NPR1, which diminished the binding of WRKY proteins, compromised the ability of NPR1 to complement the *npr1* mutation. Hence, SA affects NPR1 activity at

two stages: first, it activates NPR1 expression, and second, it stimulates the translocation of NPR1 into the nucleus where it interacts with TGA factors.

NPR1-independent SA signaling

Gene-for-gene resistance of *Arabidopsis* to Turnip crinkle virus and Cucumber mosaic virus Y are conferred by the *HYPERSENSITIVE RESPONSE TO TURNIP CRINKLE VIRUS* (*HRT*) and *RESISTANCE GENE CUCUMBER MOSAIC VIRUS Y* (*RCY1*) genes, respectively. *HRT*- and *RCY1*-conferred resistance is compromised in *NahG* plants, but not in the *npr1* mutant [36,37]. Likewise, *NahG* but not *npr1* effectively suppresses resistance gene (*R*)-conferred resistance to various *Pseudomonas syringae* strains and *Peronospora parasitica* biotypes [38,39]. These studies suggest the involvement of an NPR1-independent, SA-dependent defense mechanism in some plant–pathogen interactions (Figure 2). However, catechol, which is produced from SA by the *NahG*-encoded salicylate hydroxylase, is responsible for some of the effects of *NahG* on plant defense [40••]. Hence, evidence based solely on the analysis of *NahG* plants should be interpreted with caution. Nevertheless, the existence of an NPR1-independent mechanism is supported by studies of various *Arabidopsis* constitutive-defense-signaling mutants [12]. The *Arabidopsis suppressor of SA-insensitivity2* (*ssi2*) mutant allele confers enhanced resistance to *Pseudomonas syringae* and *Peronospora parasitica* [41]. The broad-spectrum disease resistance conferred by *ssi2* is compromised by *eds5* and *NahG* but is retained in the *npr1* mutant background. This suggests the involvement of an NPR1-independent mechanism in addition to the NPR1-dependent mechanism in *ssi2* ([41]; A Nandi, J Shah, unpublished data).

The *SSI2* gene encodes a desaturase, which primarily catalyzes the desaturation of stearic acid to oleic acid [42••]. The *ssi2*-conferred NPR1-independent resistance to *Pseudomonas syringae* is suppressed by the *suppressor of fatty-acid-desaturase deficiency1* (*sfd1*) mutant allele (A Nandi, R Welti, J Shah, unpublished data). *SFD1* encodes a glycerol-3-phosphate (G3P) dehydrogenase that synthesizes G3P for glycerolipid biosynthesis. These studies with *ssi2* and *sfd1* suggest the involvement of a lipid-derived signal(s) in *ssi2*-conferred NPR1-independent resistance. Interestingly, application of SA and BTH are not effective in activating the accumulation of *PR-1* transcripts in the *sfd1* single mutant, suggesting a role for lipid-derived signals in NPR1-dependent and -independent mechanisms (Figure 2). The common biochemical alteration brought about by the *sfd1* mutant and by mutants in the *sfd2*, *sfd3* and *sfd4* complementation groups, which also suppress *ssi2*-conferred phenotypes, is the lowered content of hexadecatrienoic acid (16:3). 16:3 and 18:3 fatty acids that are released from membrane lipids are precursors for the synthesis of oxylipins, which are potent signaling molecules [43,44•]. A α-dioxygenase (α-DOX1), which oxidizes 16- and 18-C fatty acids, has been characterized [45••]. SA activates the expression of *αDOX1*. Antisense-mediated suppression of *αDOX1* confers enhanced susceptibility to an avirulent strain of *Pseudomonas syringae*, supporting the involvement of α-DOX1 in plant defense signaling. In the past, pharmacological studies had suggested a role for lipid peroxidation in the SA-activated expression of *PR* genes [46]. Given the above evidence, it is tantalizing to speculate that fatty-acid-derived signal(s) may be involved in modulating SA-signaling in plant defense.

An NPR1-independent, SA-dependent defense mechanism is also activated in the *Arabidopsis ssi1*, *constitutive expressor of PR genes5* (*cpr5*), *cpr6* and *hypersensitive response-like lesions1* (*hrl1*) mutants [47–49]. Ethylene and jasmonic acid (JA) signaling are required for *cpr5*- and *cpr6*-conferred resistance to *Pseudomonas syringae* and *Peronospora parasitica* [48]. Likewise, ethylene is also required for the resistance conferred by *hrl1* [49]. It has been suggested that the NPR1-independent mechanism that is activated in *cpr5* and *cpr6* is akin to the local resistance activated in a leaf that is challenged with an avirulent pathogen [48]. Interestingly, the *ethylene insensitive2* (*ein2*) and *JA-insensitive1* (*jar1*) mutant alleles do not suppress the NPR1-independent, SA-dependent resistance conferred by *ssi1* [50•]. Thus, *ssi1* activates the NPR1-independent pathway at a step after the requirement for *EIN2* and *JAR1*. Alternatively, *ssi1* might activate a novel NPR1-independent mechanism.

Feedback loop(s) involving SA

Activation of *R*-gene-mediated defense signaling induces SA synthesis and downstream defense responses. Significantly, the application of SA activates the expression of *R* genes of the toll-interleukin-2 receptor (TIR)–nucleotide-binding site (NBS)–leucine-rich repeat (LRR) type [51•]. Similarly, SA activates expression of *RPW8*, which confers resistance to the powdery mildew pathogen [52]. SA also activates expression of the *EDS1* gene, which is required for SA accumulation and resistance conferred by these *R*-gene-activated pathways [21•]. This indicates feedback regulation of these *R* genes and of *EDS1* by SA (Figure 2). Likewise, expression of *EDS5* and *PAD4* genes, which regulate SA biosynthesis, and of the SA-biosynthesis gene *SID2* is activated by SA [6••,19,21•], suggesting multiple points at which SA exerts a regulatory feedback effect. Analysis of several *Arabidopsis* lesion-mimic mutants also suggests the existence of a feedback loop that involves SA in plant defense. The mechanism and biological significance of this feedback loop is not known. Activation of the expression of multiple *R* genes by SA could be part of a mechanism that activates broad-spectrum resistance. Moreover, a feedback mechanism may be important in amplifying plant defense responses, and provide a point for integrating developmental, environmental and other defense-associated signals. Indeed,

light, humidity and plant age influence SA-regulated defense responses [53–55]. In addition, studies with the JA-insensitive mutant *coronatine insensitive1* (*coi1*) demonstrate that SA synthesis/signaling is repressed by *COI1* [56•].

In contrast to the positive feedback regulation discussed above, a negative feedback loop involving NPR1 regulates SA accumulation. SA accumulation is higher in pathogen-inoculated *npr1* mutants than in wildtype plants that are inoculated with pathogen [9,11]. Likewise, SA accumulation in several lesion-mimic mutants is higher in the *npr1* mutant background than in the *NPR1* wildtype background [41,47,48]. The expression of *SID2* was also greater in pathogen-inoculated *npr1* plants than in wildtype plants [6••]. Very high levels of SA, observed in several *Arabidopsis* mutants and transgenic plants, are associated with dwarfing [18•,41,47–49]. Uncontrolled synthesis of SA may also compromise other defense pathways that are inhibited by SA [13–15]. Hence, it is important to regulate SA synthesis and signaling. Positive and negative feedback loops allow for the tighter regulation of SA accumulation and the fine-tuning of plant defense signaling.

Conclusions

SA signaling in plant defense should not be viewed as a linear pathway but rather as a complex network. Multiple stimuli can activate SA synthesis/signaling. SA can specifically bind to a variety of plant proteins affecting their activity [1,2,57•]. It can also activate gene expression/activity by multiple mechanisms and at different steps in plant defense signaling. In addition, SA influences a variety of other signaling mechanisms in plant defense [13–15]. Developmental and environmental inputs also influence SA synthesis and signaling. A feedback loop could provide a point for these diverse signals to be integrated, thus allowing the fine-tuning of SA signaling and plant defense responses. De-regulation of any of these inputs could potentially activate/repress SA-mediated defenses.

The cloning of *SID2* implies an important role for the chloroplast in the synthesis of SA. In addition, the chloroplast may also be an important location for SA action. SA-binding protein 3 of tobacco is a chloroplast-localized protein that is involved in the development of a hypersensitive response (HR) [57•]. Chloroplasts/plastids are also important for lipid metabolism and the generation of lipid-derived signals. Mutations in several genes that encode chloroplast/plastid-localized proteins alter SA synthesis and plant defense signaling [6••,42••,45••,57•,58,59], suggesting that chloroplast/plastid function/integrity may be important for the outcome of plant–pathogen interactions. Like mitochondria in animals, chloroplasts/plastids in plants might be the source of signals that affect responses to pathogens. In the future, genetic, genomic and biochemical approaches should allow further dissection of the complicated network involving SA and of the involvement of chloroplasts/plastids in plant defense.

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- •• of outstanding interest

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