Salicylic Acid, a Multifaceted Hormone to Combat Disease

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Annu. Rev. Phytopathol. 2009. 47:177–206

The *Annual Review of Phytopathology* is online at phyto.annualreviews.org

This article's doi: 10.1146/annurev.phyto.050908.135202

0066-4286/09/0908/0177$20.00

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Key Words

SA signaling, SA metabolism, systemic acquired resistance, hypersensitive response, hormonal cross talk

Abstract

For more than 200 years, the plant hormone salicylic acid (SA) has been studied for its medicinal use in humans. However, its extensive signaling role in plants, particularly in defense against pathogens, has only become evident during the past 20 years. This review surveys how SA in plants regulates both local disease resistance mechanisms, including host cell death and defense gene expression, and systemic acquired resistance (SAR). Genetic studies reveal an increasingly complex network of proteins required for SA-mediated defense signaling, and this process is amplified by several regulatory feedback loops. The interaction between the SA signaling pathway and those regulated by other plant hormones and/or defense signals is also discussed.

SA: salicylic acid
JA: jasmonic acid

EARLY HISTORY OF SALICYLATES

Plants are a rich source of natural medicines. Indeed, people often fail to realize that many currently used drugs, including digitalis, quinine, taxol, the opiates codeine and morphine, and aspirin [a synthetic derivative of salicylic acid (SA)] are derived from plants. Of these, aspirin is one of the most successful and widely used drugs. An estimated 43 million Americans, which translates into roughly one-fifth of the population, take aspirin on a regular basis. Not only does aspirin reduces pain, inflammation, and fever, but prophylactic use lowers the risk of heart attack, stroke, and certain cancers.

Long before salicylates (the general term for derivatives of SA) were identified, plants containing these compounds in large quantities were used medicinally. In the fourth century B.C., Hippocrates encouraged women to chew willow leaves to relieve the pain of childbirth (160). The use of salicylate-containing plants for therapeutic purposes continued to develop throughout the ancient world, from Rome and Asia, to the New World, where Native Americans used compresses containing extracts of willow bark to relieve pain (199). Despite the popularity of willow bark as a folk remedy, its medicinal effects were not clinically studied until the mid-1700s by the Reverend Edward Stone in Oxfordshire, England.

More than a half century later, French and German scientists competed to isolate the active ingredient in willow bark. In 1828, a German scientist, Johann A. Buchner, purified a small quantity of a yellowish substance he called salicin. Ten years later, Raffaele Piria, an Italian chemist working in Paris, split salicin into a sugar and an aromatic compound that could be converted into an acid he named acide salicylice. Other natural sources of SA were discovered around this time, but the demand for SA as a pain reliever rapidly outstripped production capability. SA was first chemically synthesized by Hermann Kolbe and coworkers in 1859; improvements to the synthetic process eventually led to large-scale production of cheaply priced SA, which led to even greater medicinal use. However, the bitter taste and unpleasant side-effects, such as chronic stomach inflammation, made long-term use of SA for conditions such as arthritis difficult. Subsequent research by Felix Hoffmann, an employee of the Bayer pharmaceutical company, revealed that acetylation of SA yielded a compound that was better tolerated, yet retained the medicinal qualities of SA. The impracticality of marketing this over-the-counter painkiller as acetylsalicylic acid led to the selection in 1899 of the trade name aspirin.

SALICYLIC ACID AS A PLANT SIGNAL/HORMONE

SA is one of a wide variety of phenolic compounds bearing a hydroxyl group or its derivative that are synthesized by plants. Traditionally, plant phenolics were classified as secondary metabolites, as they were thought to be relatively unimportant or waste products. However, this concept changed drastically with the discovery that phenolics have many important functions. For example, certain phenolics are involved in lignin biosynthesis; others serve as allelopathic compounds, regulate plant responses to abiotic stimuli, or play critical roles in plant disease resistance either by functioning as preformed or inducible antimicrobial defense compounds termed phytoalexins or by signaling defense activation (73, 124, 160). SA, in particular, influences seed germination, seedling establishment, cell growth, respiration, stomatal closure, senescence-associated gene expression, responses to abiotic stresses, basal thermotolerance, nodulation in legumes, and fruit yield (25, 88, 119, 125, 128, 145, 159, 162, 189, and references therein). Its effect on some of these processes may be indirect because SA alters the synthesis of and/or signaling by other plant hormones including jasmonic acid (JA), ethylene (ET), and auxin (see Relationship to Other Defense Signals below). In addition, SA functions as a key signal in regulating thermogenesis and disease resistance.

Flowering

The possibility that SA is an endogenous plant signal was first raised by Cleland and coworkers (reviewed in 160). By analyzing different fractions of honeydew collected from aphids feeding on vegetative or flowering *Xanthium strumarium*, they identified SA as a phloem-mobile activity capable of inducing flowering in *Lemna gibba*. Subsequent studies provided conflicting evidence regarding SA's role in flowering. However, the recent demonstrations that (*a*) SA-deficient *Arabidopsis* fail to initiate flowering in response to UV-C irradiation and flower substantially later than wild-type (wt) plants when grown under nonstress conditions (117) and (*b*) SIZ1, a SUMO E3 ligase, negatively regulates flowering via an SA-dependent pathway (77) argue that SA plays some role in this process.

Thermogenesis

Heat production, known as thermogenesis, occurs in the male reproductive structures of cycads and in the flowers of some angiosperms. In *Sauromatum guttatum* Schott (voodoo lily), an ~100-fold increase in SA precedes the onset of thermogenesis in the spadix [the central column of the inflorescence (reviewed in 88, 160)]. Exogenous SA also induces thermogenesis, and this effect is very specific: of 33 SA analogs tested, only 2,6-dihydroxybenzoic acid and aspirin induce this response. SA stimulates thermogenesis primarily by increasing the activity of the alternative respiratory pathway in mitochondria. Unlike the cytochrome respiratory pathway, electron flow through the alternative respiratory pathway generates ATP at only one site with the unused potential energy being released as heat. In voodoo lily, SA enhances the capacity of the alternative respiratory pathway by inducing expression of alternative oxidase, the terminal electron acceptor of the alternative respiratory pathway. Interestingly, SA treatment also induces alternative oxidase expression and increased alternative respiration in tobacco, a nonthermogenic plant (145 and references therein).

Disease Resistance

Shortly after the discovery that SA regulates thermogenesis in voodoo lilies, SA was shown to signal another plant process, the activation of disease resistance following pathogen infection (see sidebar, Plant Disease Resistance). Efforts to identify the signal(s) responsible for activating both local resistance and SAR have revealed important roles for SA and its derivative, methyl salicylate (MeSA). This review surveys the literature concerning the roles these signaling molecules play in activating disease resistance, covering both important earlier studies and recent findings.

SAR: systemic acquired resistance

PLANT DISEASE RESISTANCE

Often, plants recognize an invading pathogen and mount an effective defense response by means of a direct or indirect interaction between the product of a host resistance (*R*) gene and its cognate pathogen-encoded effector protein; this is termed effector-triggered immunity (ETI, formerly termed *R* gene-mediated resistance) (23, 79). If either the plant or the pathogen lacks these corresponding genes, the plant will be susceptible to infection as it cannot activate its defense responses with sufficient rapidity and/or intensity. Additionally, plants have a surface receptor-based pathogen-recognition system termed pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI, formerly called basal resistance) (23, 79), which confers low-level resistance to virulent pathogens. SA has been implicated as an important signal for the activation of both PTI (35) and ETI (37, 45, 88, 105).

One of the most visible manifestations of ETI is the hypersensitive response (HR), in which necrotic lesions develop at the site(s) of pathogen entry. ETI is usually associated with the accumulation of reactive oxygen species (ROS) and the activation of diverse groups of defense-related genes, including those encoding several families of pathogenesis-related (PR) proteins (37). A few hours to several days after HR development, the uninoculated portions of the plant often display increased levels of *PR* gene expression and the development of systemic acquired resistance (SAR), a long-lasting, broad-based resistance to infection by a wide variety of pathogens.

SALICYLIC ACID METABOLISM

SA in plants can be generated via two distinct enzymatic pathways that require the primary metabolite chorismate (reviewed in detail in 60, 215). Chorismate-derived L-phenylalanine can be converted into SA via either benzoate intermediates or coumaric acid via a series of enzymatic reactions initially catalyzed by PHENYLALANINE AMMONIA LYASE (PAL) (**Figure 1**). Chorismate can also be converted into SA via isochorismate in a two-step process involving ISOCHORISMATE SYNTHASE (ICS) and ISOCHORISMATE PYRUVATE LYASE (IPL) (**Figure 1**) (191, 200, 216): the bulk of pathogen-induced SA is synthesized via this pathway in *Arabidopsis*, *Nicotiana benthamiana*, and tomato (15, 196, 216). *Arabidopsis* encodes two ICS enzymes; SA production, as well as pathogen resistance, is severely compromised in mutants lacking functional ICS1, which appears to be responsible for approximately 90% of SA production induced by pathogens or UV light (59, 216). The appearance of residual SA in an *ics1/ics2* double mutant confirms that the *ICS* pathway is not the only source of SA in *Arabidopsis* (59).

Most of the SA produced in planta is converted into SA *O*-β-glucoside (SAG) by a pathogen-inducible SA glucosyltransferase (SAGT) (**Figure 1**) (32, 33, 34, 98, 99, 182). *Arabidopsis* encodes two SAGT enzymes; one preferentially converts SA into SAG, whereas the other forms the less abundant SA derivative, salicyloyl glucose ester (SGE) (31). SA is likely synthesized in chloroplasts (59, 191, 216) whereas tobacco SAGT appears to localize to the cytosol (33). SAG is actively transported from the cytosol into the vacuole, where it may function as an inactive storage form that can be converted back to SA (32, 33, 69).

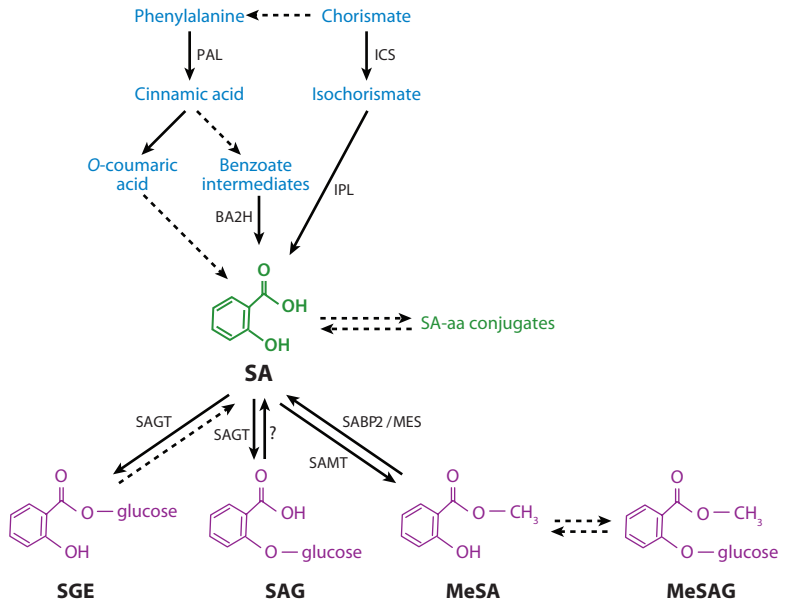

Figure 1

Simplified schematic of pathways for SA biosynthesis and metabolism as adapted from Garcion & Métraux (60). Abbreviations: PAL, phenylalanine ammonia lyase; ICS, isochorismate synthase; IPL, isochorismate pyruvate lyase; BA2H, benzoic acid-2-hydroxylase; SA, salicylic acid; SAGT, SA glucosyltransferase; aa, amino acid; SAMT, SA methyltransferase; SABP2, SA-binding protein 2; MES, methyl esterase; SGE, salicyloyl glucose ester; SAG, SA *O*-β-glucoside; MeSA, methyl salicylate; MeSAG, methyl salicylate *O*-β-glucoside.

MeSA and/or its glucosylated derivative MeSAG also accumulates to relatively high levels in vivo (**Figure 1**) (33, 34, 153, 171, 178). Radiolabeled SA is converted to almost equal amounts of SAG and MeSAG in tobacco cell suspension cultures, whereas MeSA levels are approximately five times lower (33). MeSA, like SAG, is biologically inactive (90, 171, 184). By contrast, a hydroxylated form of SA, 2,5 dihydroxybenzoic acid (gentisic acid), which also accumulates in plants (9, 31, 34), induces a specific set of *PR* genes in tomato that are not induced by SA (9).

Amino acid conjugation of SA (**Figure 1**) also may affect SA action because altered expression of acyl-adenylate/thioester-forming enzyme (GH3.5), which is involved in the conjugation of amino acids to SA and the auxin indole acetic acid (190), affects disease resistance. In one study, overexpression of *GH3.5* conferred enhanced SA accumulation, pathogen resistance, and defense gene expression (152). In a second study, *GH3.5* overexpression also resulted in elevated SA levels, but these plants displayed compromised ETI (235). However, because loss-of-function mutants were partially compromised for SAR (235), GH3.5 was proposed to be a positive regulator of SA signaling. In contrast to GH3.5, a member of subfamily II, GH3.12, a subfamily III member, was recently shown to conjugate amino acids to 4-substituted benzoates but not SA (147). Interestingly, SA inhibited this conjugation activity (147) and mutations in *GH3.12* (*pbs3/gdg1/win3*) reduced SA accumulation and pathogen resistance in *Arabidopsis* (75, 96, 144). Because SA or its functional analog benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) rescued the compromised resistance phenotypes of these *gh3.12* mutants, *GH3.12* likely acts upstream of SA in defense signaling. Okrent et al. (147) proposed that GH3.12's product, 4-hydroxybenzoate-glutamic acid, might induce or prime SA biosynthesis, with SA feedback inhibiting GH3.12's activity and thereby modulating its own synthesis.

DISEASE RESISTANCE

Salicylic Acid is an Endogenous Resistance Signal

ETI: effector-triggered immunity

HR: hypersensitive response

A possible role for SA in signaling disease resistance was first suggested by White and coworkers, who demonstrated that injecting leaves of resistant tobacco with SA or aspirin stimulated PR protein accumulation and enhanced resistance to tobacco mosaic virus (TMV) infection, manifested by a 90% reduction in lesion number (reviewed in 88, 113, 160). SA treatment was subsequently found to induce *PR* gene expression and/or resistance to viral, bacterial, and fungal pathogens in many plant species. Furthermore, it induced the same set of genes in tobacco and *Arabidopsis* as was activated during SAR.

SA was initially proposed to act by mimicking an endogenous phenolic signal for resistance; however, analyses of SA levels in cucumber and tobacco argued that it was the actual defense signal (for reviews on SA's role in resistance, see 37, 45, 88, 105, 113, 160). In TMV-resistant (but not TMV-susceptible) tobacco, SA levels increased more than 20-fold in the inoculated leaves and over 5-fold in the systemic leaves; in both sets of leaves these increases preceded or paralleled *PR* gene expression (111). Similarly, SA levels increased 10- to 100-fold in the phloem exudates of cucumber inoculated with tobacco necrosis virus, *Colletotrichum lagenarium*, or *Pseudomonas syringae*, and these increases preceded SAR development and induction of a defense-associated peroxidase activity (123, 161, 181). Additional evidence supporting a signaling role for SA came from the demonstration that high temperature growth conditions (>28°C) suppressed disease resistance (including HR development), *PR* expression and SA accumulation in TMV-resistant tobacco (112, 219). Shifting these plants to room temperature led to a dramatic increase in SA levels, which preceded lesion formation and *PR-1* expression. Furthermore, the highly elevated SA levels found in certain plants and genetic mutants correlated with their enhanced resistance to pathogen infection.

The strongest evidence supporting SA's role as a critical defense signal has come from analyses of plants in which endogenous SA levels were altered. The first of these studies utilized transgenic tobacco or *Arabidopsis* expressing the bacterial *nahG* gene, encoding the SA-metabolizing enzyme salicylate hydroxylase. Following pathogen infection, these plants were unable to accumulate high SA levels, and they failed to develop SAR or express *PR* genes in the systemic leaves; instead, they displayed heightened susceptibility to both virulent and avirulent pathogens (36, 58, 94, 201). Both disease resistance and *PR* expression were restored in these plants by treatment with the SA synthetic analog, 2,6-dichloro-isonicotinic acid (INA) (36, 202). Subsequent studies revealed that plants defective for SA biosynthesis displayed a similar phenotype. Tobacco or *Arabidopsis* with suppressed *PAL* expression or mutations in *SID2/EDS16* (encodes ICS1) or *SID1/EDS5* (encodes a member of the MATE transporter family required for SA accumulation) displayed enhanced pathogen susceptibility and/or failed to develop SAR or systemically express *PR* genes (141, 142, 149, 216). Like *nahG* plants, resistance and *PR* expression were restored by treatment with SA or INA. Overexpression of enzymes involved in SA metabolism, including SA glucosyltransferase 1 (AtSGT1) or SA methyltransferase (OsBSMT1), in transgenic *Arabidopsis* also led to reduced endogenous SA levels, reduced *PR* expression and enhanced susceptibility to pathogens (90, 184). By contrast, overexpression of bacterial SA biosynthetic genes in transgenic tobacco conferred highly elevated SA levels, constitutive *PR* expression, and enhanced resistance (200).

Although SA's role as a defense signal is well established in dicots, its role in monocots is less well understood. SA or its synthetic analogs INA or BTH induce *PR* expression and/or resistance in maize (127), rice (74, 167, 170), barley (89, 136), and wheat (3, 65). In addition, endogenous SA levels increase in barley resisting infection by the bacterial pathogen *P. syringae* pv. *syringae* (198). However, in near isogenic lines of barley carrying the powdery mildew resistance genes *mlo5*, *Mlg*, or *Mla12*, defense responses (including HR development and H_2O_2 accumulation) were activated after pathogen infection without a corresponding rise in SA levels (72). SA levels similarly failed to increase in rice inoculated with *P. syringae* or the fungal pathogens *Magnaporthe grisea* (causal agent of rice blast) or *Rhizoctonia solani* (179), and pathogen-induced *PR* expression was unaffected in SA-deficient, *nahG*-expressing transgenic rice (222). However, because rice has constitutively high levels of SA, signaling might occur via altered sensitivity of downstream components to SA, rather than via actual changes in SA levels (20). Supporting a role for SA as a defense signal in rice are the combined findings that (*a*) *nahG*-expressing rice exhibits increased susceptibility to *M. grisea* (222), (*b*) overexpression of *NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES 1* (*NPR1*, a critical SA signal transducer in *Arabidopsis* and tobacco) or its rice homolog enhances resistance to the bacterial blight-causing *Xanthomonas oryzae* pv. *oryzae* (21, 22, 226), (*c*) rice/*Arabidopsis* NPR1 interacts with rice TGA transcription factors, one of which binds a *cis*-element required for SA responsiveness in the rice *RCH10* promoter (21, 22), (*d*) SA or BTH treatment induces expression of several rice *WRKY* genes (members of the *Arabidopsis* WRKY transcription factor family participate in defense responses) (104, 175) and (*e*) expression of *WRKY45* is required for BTH-induced resistance to blast disease (175). Recent studies have provided hints that SA may also mediate defense signaling in wheat. Not only do elevated SA levels correlate with constitutive *PR* expression in a wheat lesion-mimic mutant (3) but wheat overexpressing *Arabidopsis NPR1* also displays enhanced resistance to *Fusarium graminearum* (110). Taken together, these results suggest that SA is a signal for defense responses in at least some monocots and that many of the components involved in this pathway(s) are analogous to those utilized in dicots.

Salicylic Acid and Systemic Acquired Resistance

For SAR to develop in systemic leaves, a signal generated in the inoculated leaf is transmitted via the phloem to the uninfected portions of the plant (37, 45, 46, 88, 113, 203). SA was initially proposed to serve this function because (*a*) SA levels rise coincidently with or just prior to SAR development and systemic *PR* expression or peroxidase activation in pathogen-infected tobacco and cucumber (111, 123, 161), (*b*) SA was detected in the phloem of pathogen-infected cucumber and tobacco (123, 126, 161, 219), and (*c*) radio-tracer studies suggested that a significant amount of SA in the systemic leaves of pathogen-infected tobacco and cucumber was transported from the inoculated leaf (126, 177). However, leaf detachment assays suggested that the mobile SAR signal moved out of the infected leaf before increased SA levels were detected in petiole exudates from that leaf (161, 181). Additionally, SA-deficient, *nahG*-expressing (or PAL-suppressed) rootstock leaves of chimeric tobacco generated a signal after TMV infection that activated SAR and systemic *PR* expression in wt scion leaves (149, 201). The possibility that small amounts of SA generated in these *nahG*-expressing or PAL-suppressed rootstock leaves were sufficient to induce SAR was discounted by analyses of transgenic tobacco expressing the A1 subunit of cholera toxin (CTX) (8). These plants accumulated elevated SA levels, constitutively expressed *PR* genes, and exhibited enhanced resistance; however, CTX-expressing rootstocks did not induce SAR in the wt scions of chimeric tobacco.

To reveal the role of SA in defense signaling, several tobacco SA-binding proteins were identified. Of these, SA-binding protein 2 (SABP2) was required for both local resistance and SAR following TMV infection (93). Because SABP2 displays SA-inhibitable methyl salicylate esterase activity (**Figures 1** and **2**) (54), it was proposed that SABP2 functions by converting MeSA, which is biologically inactive (90, 171), to SA, which in turn downregulates SABP2 activity. Grafting analyses demonstrated that SABP2's esterase activity is required only in the systemic tissues of TMV-infected tobacco plants for SAR development (153). SAR was abolished when MeSA accumulation was repressed in the SAR signal-generating leaves by either silencing *SA methyltransferase 1*, which generates MeSA from SA (**Figure 1**), or overexpressing a mutant SABP2 whose MeSA esterase is not inhibited by SA. These results, together with the rise in MeSA levels in petiole exudates of TMV-infected leaves, indicate that MeSA is a phloem-mobile signal for SAR (**Figure 2**). Further studies in *Arabidopsis* (204) and potato (P. Manosalva, S.W. Park, & D.F. Klessig, unpublished results) suggest that the roles of MeSA and its esterases in SAR are conserved. It should be noted that MeSA is one of several likely long-distance signals for SAR; other possible signals are reviewed elsewhere (203). MeSA also may function as an airborne defense signal; MeSA emitted from TMV-infected tobacco or *P. syringae*-infected *Arabidopsis* expressing *OsBSMT1* (a SA/BA methyltransferase gene from rice), induced defense gene expression in neighboring plants (90, 178). Also, high levels of MeSA were detected in a forest canopy in response to ecosystem-scale abiotic stresses (84).

Salicylic Acid-Mediated Signaling

A complex genetic regulatory network that either affects signaling upstream of SA or is required to relay the disease resistance signal downstream of SA has been uncovered. Here, we discuss current knowledge of SA-mediated signaling, including upstream and downstream signals and pathways, as well as knowledge gained from constitutive defense response mutants.

Upstream signaling. The lipase-like protein ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) and its sequence-related interacting partner PHYTOALEXIN DEFICIENT 4 (PAD4) act upstream of SA in basal resistance to host-adapted biotrophic pathogens as well as in ETI initiated by the TIR-NBS-LRR subset of *R* genes (1, 213). SA can

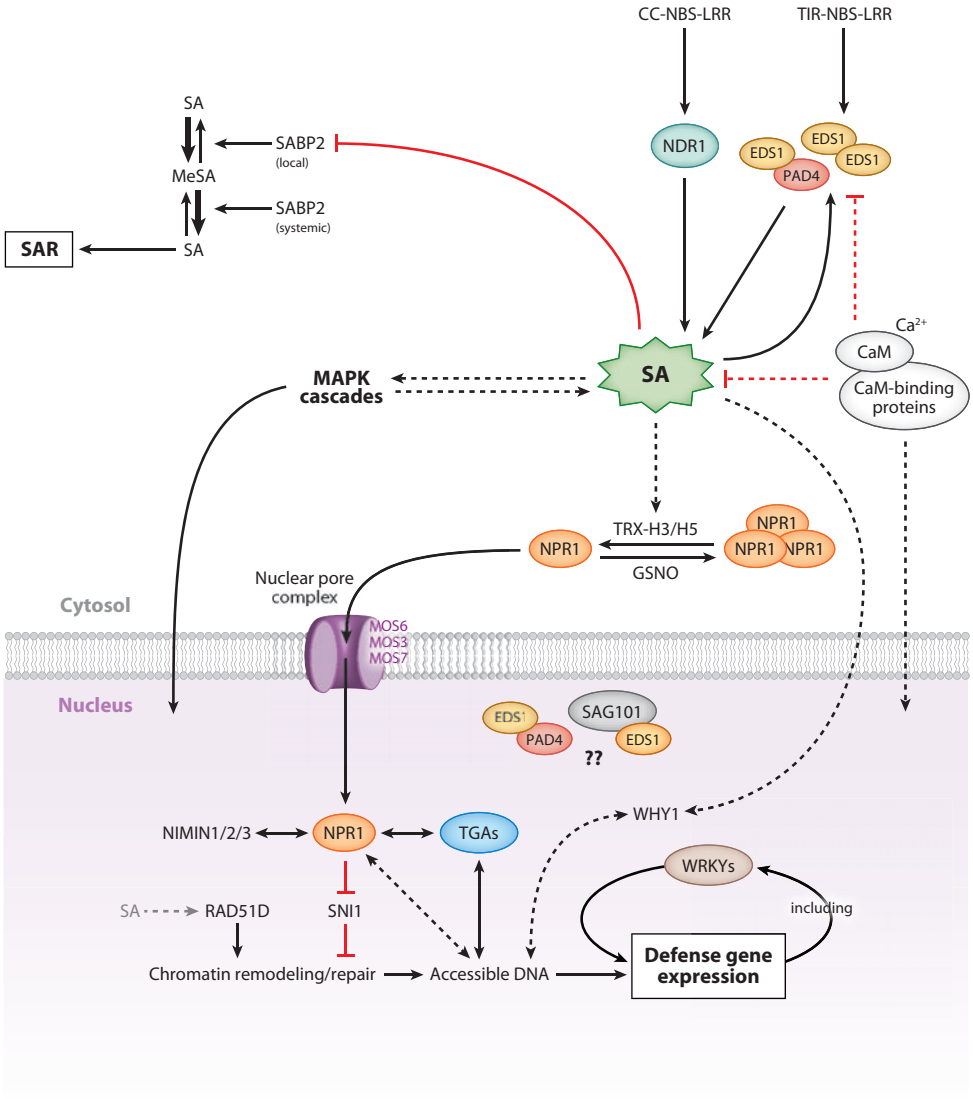

Figure 2

Schematic overview of SA signaling as summarized in Disease Resistance. Arrows indicate activation of enzymatic activities, induction or accumulation of compounds or gene transcripts or, in the case of NPR1, movement of the NPR1 monomer from the cytosol into the nucleus. Double-headed arrows indicate physical protein-protein or protein-DNA interactions, whereas red lines indicate repression of enzymatic activities or accumulation of compounds. Solid lines indicate established interactions; dashed lines represent hypothesized or less well characterized interactions. The grey dashed line indicates a direct or indirect activation of RAD51D by SA.

rescue defense gene induction in *eds1* and *pad4* mutants and induce expression of *EDS1* and *PAD4* in wt plants (50, 52, 237), arguing that EDS1 and PAD4 lie upstream of SA and are positively feedback regulated by SA (**Figure 2**). This positive feedback loop likely potentiates SA action and may be regulated by different EDS1 complexes, including cytosolic EDS1

homodimers, nucleo-cytoplasmic EDS1-PAD4 heterodimers and nuclear interactions between EDS1 and the PAD4-related and partly redundant SENESCENCE-ASSOCIATED GENE 101 (SAG101) protein (**Figure 2**) (53). EDS1 is needed for PAD4 and SAG101 accumulation, and analysis of mutant combinations points to a cooperative signaling role of all three partners in host defense (53). Trafficking of EDS1 complexes between the nucleus and cytoplasm appears to be important for effective disease resistance signaling (53, 214; Cheng Y, Germain H, Wiermer M, Bi D, Xu F, Garcia A, Wirthmueller L, Despres C, Parker J, Zhang Y & Li X, manuscript in review).

Signaling downstream from the second major subset of R proteins, CC-NBS-LRR type, is generally regulated by *NONSPECIFIC DISEASE RESISTANCE 1* (*NDR1*) rather than by *EDS1* (**Figure 2**) (1, 16). BTH rescued the SAR-deficient phenotype of *ndr1* mutant *Arabidopsis*, indicating that NDR1 acts upstream of SA (174). *ndr1* exhibited suppressed PTI and ETI, whereas overexpression of *NDR1* significantly reduced growth of virulent bacteria (28, 174). NDR1 is a glycophosphatidyl-inositol-anchored plasma membrane protein (28) that may regulate R protein signaling via a physical interaction with RPM1-INTERACTING PROTEIN 4 (RIN4) (29, 79).

Two recent studies revealed a prominent role for Ca^{2+} and calmodulin (CaM) in the regulation of SA accumulation and signaling. Binding of Ca^{2+}/CaM to the *Arabidopsis* transcription factor SR1 represses expression of *EDS1* and suppresses SA accumulation and defense (**Figure 2**) (44). Conversely, binding of CaM to PTI-induced CaM-binding protein 60-like.g is positively correlated with SA accumulation and pathogen resistance (208).

Downstream signaling. Signaling mechanisms leading to defense gene induction downstream of SA include SA-protein and protein-protein interactions as well as genetic interactions; these are discussed below. SA-induced changes in gene expression also are discussed.

Biochemistry: SA-binding proteins. A receptor for SA has been long sought, and four tobacco proteins that bind SA have been identified. Of these, SABP2 has the highest affinity for SA (43). Binding of SA to SABP2's active site feedback inhibits SABP2's MeSA esterase activity and facilitates MeSA accumulation in the infected tissue for transport to uninfected tissue (54, 153). Two other SA-binding proteins suggest a role for reactive oxygen species (ROS) in SA signaling. The first SABP identified was catalase (CAT); its H_2O_2-degrading activity is specifically inhibited by SA or biologically active SA analogs (**Figure 3**) (18). SA also inhibits the H_2O_2-scavenging activity of cytosolic ascorbate peroxidase (APX), and H_2O_2 levels concomitantly rise upon SA treatment of tobacco leaves (reviewed in 37). Thus, SA may facilitate H_2O_2 accumulation during the oxidative burst (OB) induced by infection with avirulent pathogens. Further interactions between SA and ROS are discussed in Relationship to Other Defense Signals below. Another SA-binding protein, SABP3, is tobacco chloroplastic carbonic anhydrase (CA) (180). Although CA appeared to have antioxidant activity in yeast (66), this conclusion was called into question (24). Nonetheless, silencing *CA* in *Nicotiana benthamiana* suppressed Pto:avrPto-mediated HR (180) and increased susceptibility to *Phytophthora infestans* (164), thus arguing that this enzyme plays a role in plant immunity. Recently, Wang et al. (206) demonstrated that CA is S-nitrosylated, and this suppresses its SA binding and enzymatic activities. Because CA enzymatic activity is required for resistance, S-nitrosylation could be part of a negative feedback loop that modulates the defense response.

NPR1 and its interacting proteins. Signaling downstream from SA is largely regulated via *NPR1*, also known as *NIM1* or *SAI1* (**Figure 2**) (reviewed in 42, 46, 155). NPR1 contains an ankyrin-repeat motif and a BTB/POZ domain. The protein occurs in the nucleus, where it functions in SA-mediated *PR-1* gene induction, and in the cytosol, where it plays a role in antagonistic cross talk between SA and JA (188; see

PTI: PAMP-triggered immunity

ROS: reactive oxygen species

OB: oxidative burst

Relationship to Other Defense Signals below). Cytosolic NPR1 oligomers are held together by disulphide bridges and monomerize upon SA-induced changes in the cellular redox state leading to reduction of two cysteine residues (Cys82 and Cys216) by THIOREDOXIN-H5 (TRX-H5) and/or TRX-H3 (130, 192). NPR1 monomers are subsequently translocated from the cytosol into the nucleus, where they activate defense gene transcription (**Figure 2**); mutation of either Cys82 or Cys216 elevated the level of monomeric, nuclear localized NPR1, which correlated with constitutive upregulation of *PR-1* expression (130). Moreover, a mutant form of NPR1 that is constitutively monomeric and present in the nucleus was recently shown to enhance resistance (192). However, plants expressing only this mutant form of NPR1 were incapable of mounting an SA-dependent SAR response owing to rapid degradation of NPR1. This finding suggests that both monomerization and (re)oligomerization of NPR1 are required for the full array of SA-mediated resistance mechanisms and provides a further link between SA and redox signaling because (re)oligomerization is facilitated by S-nitrosylation of Cys 156 of NPR1 (**Figure 2**) (192).

Yeast two-hybrid screens have revealed direct interactions between NPR1 and several members of the TGA family of bZIP transcription factors, as well as with three other proteins, NIMIN1, 2, and 3 (NIM1-interacting1, 2, 3) (**Figure 2**) (209, 210, reviewed in 46). Although *NIMIN1*, *2*, and *3* are induced transiently relatively early after SA treatment of *Arabidopsis* (209), NIMIN1 appears to negatively regulate SA/NPR1 signaling; overexpression of *NIMIN1* resulted in compromised ETI and SAR, whereas reduced expression of the same gene enhanced induction of *PR-1* expression by SA (210).

NPR1 interacts with five different *Arabidopsis* TGA factors in yeast (46) and up to seven in plants (86 and references therein). In planta interaction between NPR1 and TGA1 or TGA4 was detected only upon SA treatment of leaves. The interaction depended on SA-induced changes to the redox environment that resulted in the reduction of two cysteine residues that are conserved in TGA1 and TGA4 (46). In planta interaction between TGA2 and NPR1 can be detected in the absence of SA, but is enhanced by SA treatment of leaves (51), whereas the ability of TGA2 and TGA3 to activate transcription requires SA and NPR1 (46, 166). Because NIMIN1 can form a complex with TGA2 or TGA6, NPR1, and a *PR-1* promoter element in yeast (210), it might modulate TGA-dependent transcriptional activation of SA-regulated genes (**Figure 2**).

Gene induction: immediate-early/late. Genes regulated downstream of SA can be divided roughly into two classes: immediate-early genes, induced within 30 minutes of SA treatment (71), and genes induced later, including the SA-marker gene *PR-1* (95). Gene expression profiling experiments conducted over the past ten years (reviewed in 48) have revealed complex patterns of regulation for large numbers of genes that are induced or repressed relatively early (e.g., 6, 11) or late (e.g., 6, 62, 114, 169) after SA treatment (11, 169) or pathogen infection (6, 62). By comparing the transcript profiles of wt plants with those of SA signaling or biosynthesis mutants, including *ics1* (17), different clusters of genes whose expression is modulated by SA were identified. The multitude of differentially SA-regulated genes with predicted functions in defense, metabolism, and development allowed a first glance into the complexity of SA interactions with other signaling pathways (see also Relationship to Other Defense Signals below).

Unlike late SA-responsive genes, expression of immediate-early genes, including *GLUTATHIONE S TRANSFERASE 6* (*GST6*) and *IMMEDIATE EARLY-INDUCED GLUCOSYLTRANSFERASE* (*IEGT*), does not require de novo protein synthesis (197 and references therein). Also, transcript profiling analyses indicate that genes induced very early do not require functional NPR1. However, *NPR1* dependence for induction of genes progressively increases over time (10, 11).

SA-mediated induction of the *NPR1*-independent, immediate-early genes tested depends at least partially on TGA2 in *Arabidopsis* and tobacco (10, 194). Consistent with this finding, the *as-1* promoter element, which is recognized by TGA factors, is found in the promoters of immediate-early SA-induced genes in both species.

The best studied late SA-inducible gene is *PR-1*. Its promoter contains one negative regulatory element and at least one positive element, including the *as-1* element that is bound by TGAs (95). Four of the TGA factors that interact with NPR1 differentially regulate *PR-1* expression in *Arabidopsis* (86). TGA3 enhances SA-induced *PR-1* expression, based on reduced levels of the transcript in SA-treated *tga3* (86). SA-inducible transcription of *PR-1* also is fully abolished in a *tga2tga5tga6* triple mutant, but not in any of the single or double mutants tested, indicating that TGA2, -5, and -6 are functionally redundant positive regulators of SA-dependent *PR-1* expression (reviewed in 46). However, because basal *PR-1* levels are elevated in the *tga2tga5tga6* triple mutant in a *TGA3*-dependent manner, TGA2, -5, and -6 repress *PR-1* transcription in the absence of SA (46, 86). Consistent with this latter finding, TGA2 acts as a transcriptional repressor in *Arabidopsis* (166), and TGA factors in tobacco have both negative and positive roles in regulating defenses (156). Although several studies have concluded that NPR1 and SA enhance binding of TGA2 to *PR-1* promoter elements (46), Rochon et al. (166) argued that NPR1 and TGA2 are independently recruited to the *PR-1* promoter, but that SA enhances the interaction between NPR1 and TGA2, resulting in the formation of a so-called enhanceosome, in which the C terminus of NPR1 functions as a transcriptional transactivator (**Figure 2**). However, the precise mechanism by which NPR1 cooperates with TGA2 to induce defense gene expression remains unclear, as data of Johnson et al. (78) recently suggested that transient binding of NPR1 to TGA2 acts to promote dimerization and DNA binding of TGA2, upon which NPR1 dissociates from the complex.

In addition to TGAs, several members of the WKRY family of transcription factors differentially regulate SA signaling (reviewed in 48, 49). BTH-inducible expression of eight *WRKY* genes is controlled by NPR1 and by TGA2, -3, -5, and/or -6 (**Figure 2**) (205). Analyses of single and double mutant phenotypes of *NPR1*-regulated *WRKY* genes showed that WRKY18, -53, -54, and -70 are positive regulators of SA-mediated resistance, and the compromised resistance phenotype of *wrky18* is dependent on the negative regulator WRKY58. It should be noted that the positive effect of WRKY18 on SA signaling appears to be limited to SA-inducible resistance or SAR because PTI to several pathogens is enhanced in *wrky18* (49, 205, 218). The latter effect appears to be at least partly dependent on WRKY18 functional homologs and interacting partners WRKY40 and/or WRKY60 (218). WRKY70 is required for SA-mediated resistance in various plant-pathogen interactions (49, 100, 101), although expression of *ICS1* and SA accumulation were upregulated in *wrky70/wrky54* double mutants (205). This result indicates that these orthologous WRKYs might help orchestrate feedback control of the SA signaling pathway in addition to enhancing SA-dependent resistance (205). Most of the WRKY factors discussed above, including WRKY18 and 70, have opposing effects on SA and JA signaling, thereby forming prominent points of antagonistic cross talk between these signaling pathways (49, 100, 101, 218).

Several SA- or pathogen-induced WRKY factors, including WRKY7, -11, -17, -38, and -62, repress SA-mediated defense (49, 87). *WRKY38* and *62*, which are both induced by SA in an *NPR1*-dependent manner, encode negative regulators of PTI whose activities are regulated by HISTONE DEACETYLASE 19 (HDA19) (87). WRKY38 and WRKY62 physically interact with HDA19 in the nucleus and, in an in planta transcription assay, WRKY38 and WRKY62 act as transcriptional activators whose activity is repressed by HDA19. Thus, HDA19 may positively regulate PTI by repressing WRKY38 and WRKY62, which indirectly

suppress resistance by activating an unknown repressor (87). Together with SA, MeJA further induces expression of *WRKY62*, and this induction was shown to depend on SA and on (cytosolic) NPR1 (115). As both SA- and JA-dependent gene expression negatively correlates with the expression level of *WRKY62* (87, 115), the data indicate that WRKY62 and/or WRKY38 affect synergistic cross talk to repress both SA and JA signaling.

The ability of SA to induce gene expression via an *NPR1*-independent pathway may require members of the WHIRLY (WHY) transcription factor family; these proteins bind single-stranded DNA in an *NPR1*-independent fashion (39, 40). DNA-binding activity of *St*WHY1 or *At*WHY1 is induced by arachidonic acid or SA, respectively (40), and *StPR-10a* expression correlates with *St*WHY1 DNA-binding activity (38). *Atwhy1* mutants with compromised DNA-binding activity display enhanced susceptibility to virulent and avirulent strains of *Hyaloperonospora parasitica* (40). Because SA-induced *PR-1* expression and resistance are abolished in *Atwhy1*, even though the WHY1-binding PB promoter element is not found in the *PR-1* promoter, Desveaux et al. (39, 40) proposed that WHY1 and NPR1 cooperate in parallel to relay the SA signal.

Other transcription factors that affect SA signaling, including ethylene response factors (ERFs) and R2R3-MYB factors, are discussed in more detail by Eulgem (48). *At*MYB30, for instance, positively regulates the pathogen-induced HR in an SA-dependent, *NPR1*-independent manner (158), whereas *At*bZIP10, a member of the *At*bZIP family of transcription factors, positively regulates the HR through a mechanism that may depend on both SA and *NPR1* (5, 83). Potential roles of SA in HR cell death are discussed below (Relationship to Other Defense Signals).

Genetics/mutants: npr1 suppressors. SA signaling coincident with or downstream from NPR1 has been further investigated using various suppressor and suppressor-of-suppressor mutants (47, 102, 103, 172, 234). One mutation suppressing the *npr1* mutant phenotype, *ssi2* (*suppressor of SA insensitivity 2*), occurs in a gene encoding a stearoyl-ACP desaturase (81). *ssi2* constitutively accumulates SA and *PR-1* transcripts and displays enhanced resistance to biotrophic pathogens (81, 82, 138, 173). This phenotype partially requires SA, *EDS1*, and *PAD4*, but not *NPR1*. Because *ssi2*-mediated resistance also depends on genes affecting chloroplastic galactolipid metabolism (80, 82, 137), the combined data indicate that galactolipid metabolism affects SA-mediated defense via an *NPR1*-independent mechanism. Furthermore, *SSI2* appears to constitute a node of antagonistic cross talk between JA and SA because JA-induced *PDF1.2* expression and resistance to necrotrophic *Botrytis cinerea* are repressed in *ssi2* (81, 138, 173). However, the positive effect of *SSI2* on JA signaling appears to operate through different genetic components than its repressive role in SA signaling because SA, *EDS1*, and *PAD4* are largely dispensable for repression of *PDF1.2* expression in *ssi2* (82, 138).

Two different *npr1*-suppressor mutants, *ssi4* and *snc1* (*suppressor of npr1-1 constitutive1*), display constitutive SA-dependent, *NPR1*-independent resistance owing to mutations in the encoded R proteins (102, 176, 229). Both mutants constitutively accumulate SA and express *PR-1*. In turn, several mutations suppressing the constitutive resistance phenotype of *snc1* were identified. *MODIFIER OF snc1* (*MOS*)2 encodes a nuclear protein with putative RNA-binding motifs (227), and MOS5 encodes ubiquitin-activating enzyme UBA1; this latter finding implicates ubiquitination in R protein-mediated signaling (64). The *MOS3* and 7 genes encode nucleoporins, and *MOS6* encodes importin α3 (150, 214, 234; Cheng Y, Germain H, Wiermer M, Bi D, Xu F, Garcia A, Wirthmueller L, Despres C, Parker J, Zhang Y & Li X, manuscript in review). Mutations in *MOS3* or *6* compromised SA-dependent resistance as well as *snc1*-dependent SA accumulation (150, 234), reinforcing the importance of nucleo-cytoplasmic trafficking of proteins, such as NPR1, various R proteins,

and possibly EDS1, in SA-mediated signaling (**Figure 2**) (reviewed in 214).

Within the nucleus, chromatin remodeling and/or DNA recombination were recently implicated in both *NPR1*-dependent and-independent, SA-dependent defense gene induction (47, 116, 129). Mutations in genes encoding histone H2A.Z homologs or members of the corresponding chromatin remodeling complex in *Arabidopsis* conferred heightened SA-dependent defense gene expression and resistance (116). Also, *SUPPRESSOR OF npr1-1 INDUCIBLE 1* (*SNI1*) encodes a protein that normally represses *NPR1*-dependent defense gene expression until it is itself repressed by NPR1 upon induction of SA signaling (103, 129). Constitutive derepression of defense gene transcription in *sni1* is suppressed by a mutation in *RAD51D*, which encodes a protein involved in DNA recombination and repair (47). Therefore, Durrant et al. (47) hypothesized that SA regulates the accessibility of DNA for transcription through NPR1, SNI1, and/or RAD51D (**Figure 2**). To add a layer of complexity, *NPR1*-independent signaling downstream from *snc1* depends on a complex of at least three proteins that shows homology with mammalian protein complexes involved in RNA splicing (151). Although these data imply a regulatory role for RNA splicing in SA-mediated resistance, alternative splicing of several tested gene transcripts, including that for the *R* gene *RPS4*, was unchanged in all the three potential splicing mutants that show repressed *NPR1*-independent defense (151).

Constitutive defense response mutants. Many stimuli, including UV light, ozone, and the repression/inappropriate expression of foreign or endogenous genes, lead to constitutive activation of defense responses and, in some cases, spontaneous cell death (45). However, it remains unclear whether these stimuli induce defenses by activating a resistance pathway or whether they simply cause metabolic stress, which leads to elevated SA levels. An assortment of *Arabidopsis* mutants that display constitutive SA accumulation, *PR* gene expression, and SAR have been identified. The morphology of many constitutive defense mutants is altered, as their growth is stunted and their leaves are curly (46); some, termed lesion mimic mutants, develop uncontrolled or spontaneous HR-like cell death (106). Owing to the large number and diversity of genes whose mutation confers constitutive defense activation, this section only focuses on recent findings. Please note that several constitutive defense mutants have been discussed previously, including *H2A.Z*, the *npr1* suppressors *ssi2*, *ssi4*, *snc1*, and *sni1*; the MAPK-associated mutants *mpk4*, *edr1*, and overexpressors of *MKK7* or *MKS1* are discussed in Relationship to Other Defense Signals.

Several studies have indicated that environmental conditions regulate the phenotype displayed by certain constitutive defense mutants. High relative humidity growth conditions suppress both the dwarf morphology and enhanced disease resistance phenotype in *lsd6* (212), *cpr22* [a novel cyclic nucleotide-gated ion channel (224, 225)], *ssi4* (176, 236), *slh1* [a TIR-NBS-LRR-WRKY type R gene (146)] and *cpn1/bon1* [a copine (76, 220)]. SA accumulation in several of these mutants is suppressed by high humidity growth conditions, underscoring the correlation between SA and the constitutive defense phenotype (146, 224, 236). Light intensity or duration also affects the phenotype of some constitutive defense mutants. High light intensity largely suppresses the dwarf phenotype of *cpr1-1*, *cpr5-1*, *cpr6-1*, and *dnd1-1* (118), whereas continuous light induces the stunted phenotype of *bap1* [a membrane-associated phospholipid-binding protein that interacts with *cpn1/bon1* to negatively regulate *snc1* (221)]. Coronatine-induced cell death in *acd2* (red chlorophyll catabolite reductase) also requires light (109), and long-day or high-intensity light triggers runaway cell death in *lsd1* [a novel zinc finger protein (119)]. *lsd1* appears to condition runaway cell death because of a failure to acclimate to light conditions that promote excess elicitation energy (EEE) (119). EEE, which is the amount of absorbed light energy in excess of what is needed for photosynthesis, is promoted in wt plants by rapid

changes in light intensity or quality. In *Arabidopsis*, EEE induces stomatal closure, which in turn activates photorespiration and thus H_2O_2 production; it also induces cell death and expression of *APX2* and *PR-1* and stimulates SAR and PTI (131). Strikingly, SA inhibits acclimation to EEE-promoting conditions, as it induces stomatal closure, reduces photosynthetic electron transport, and leads to photobleaching and cell death (119). Because stomatal closure is inversely proportional to relative humidity, the ability of low humidity or high-intensity light to induce cell death and/or stunting in some constitutive defense mutants may have a common mechanism in the generation of EEE-associated redox stress.

Analyses of various defense mutants have led to the suggestion that an additional signal(s) besides SA is required to promote disease resistance and HR development. Supporting this possibility, *nahG*, *sid2-2*, and *eds5-1* suppressed the stunted, necrotic phenotype of *acd11* to different extents, although SA levels in *acd11/nahG* and *acd11/sid2-2* plants were comparable (13). This finding, combined with the inability of SA to restore cell death in *acd11/sid2-2* plants, led to the suggestion that two groups of compounds, one isochorismate derived and the other salicylate hydroxylase degradable, work with SA to promote some *acd11* phenotypes, including spontaneous cell death. The existence of an additional signal also could explain why *eds5-3*, *sid2-1*, or *nahG* only partially rescued the dwarf, necrotic phenotype displayed by *syp121/122*, which contains a double mutation in syntaxins [proteins essential for the SNARE machinery that controls vesicle traffic (230, 233)].

Analyses of other constitutive defense mutants have suggested that an amino acid-derived molecule(s) regulates resistance signaling at a point upstream of SA. Mutations in the aminotransferases encoded by *AGD2* and *ALD1* confer enhanced disease resistance and enhanced pathogen susceptibility, respectively; thus, an AGD2-generated molecule may suppress defenses, whereas that synthesized by ALD1 might activate them (185). Modification of an unknown target protein with a small ubiquitin-like modifier (SUMO) peptide also appears to regulate defenses, as the *siz1* mutant (defective for a SUMO E3 ligase) displays SA-dependent enhanced resistance mediated by TIR-NBS-LRR-type but not CC-NBS-LRR-type R proteins (97). The combined observations that (*a*) EDS1 and PAD4 contain potential sumoylation sites, (*b*) both are required for TIR-NBS-LRR-mediated signaling, (*c*) they may regulate glycerol metabolism, and (*d*) glycerol induces SA accumulation led to the suggestion that SIZ1-mediated sumoylation of EDS1 and PAD4 represses their activity in glycerol metabolism, thereby downregulating SA biosynthesis.

RELATIONSHIP TO OTHER DEFENSE SIGNALS

MAPK Signaling

Mitogen-activated protein kinase (MAPK or MPK) cascades are involved in many signal transduction pathways in plants, as well as in mammals and fungi. In *Arabidopsis*, stress signaling mainly depends on AtMPK3, -4, and -6, with SA signaling positively regulated via AtMPK3 and AtMPK6 and negatively regulated via AtMPK4 (reviewed in 26). A connection between SA and plant MAPKs was first made in 1997 with the purification and initial characterization of the tobacco SA-INDUCED PROTEIN KINASE (SIPK) (231). SIPK (and its *Arabidopsis* ortholog AtMPK6) and the tobacco WOUND-INDUCED PROTEIN KINASE (WIPK, and its *Arabidopsis* ortholog AtMPK3) were shown to play important roles in responses to biotic stresses, such as infection, and abiotic stimuli, such as wounding, cold, drought, osmolarity, UV irradiation and ozone (reviewed in 232). Silencing of *AtMPK6* compromised PTI and ETI to biotrophic pathogens (122). Additionally, AtMPK3, and to a lesser extent MPK6, was shown to play a pivotal role in SAR and SA-mediated priming of plants for disease resistance (7). Priming with low concentrations of SA or BTH results in the induction of a highly efficient defense response to

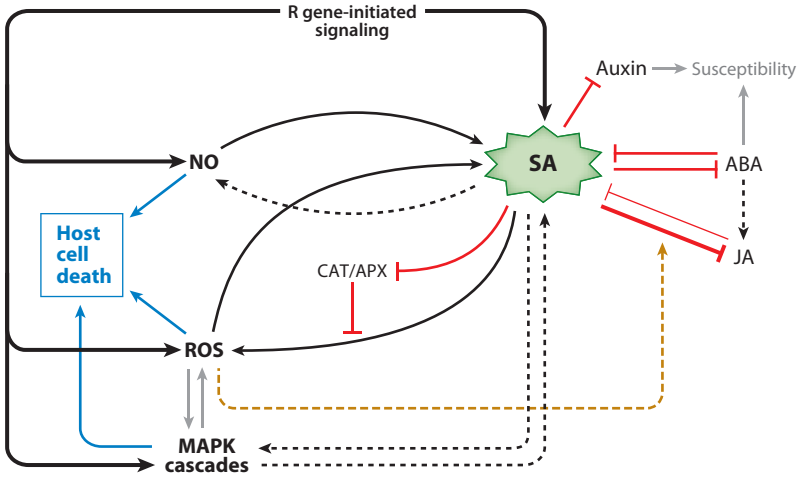

Figure 3

SA in relation to other defense signals. Arrows indicate activation, whereas red lines indicate repression. Solid lines indicate established interactions; dashed lines represent hypothesized or less well characterized interactions. Interactions in grey are not discussed in detail in this review. Abbreviations: ABA, abscisic acid; APX, ascorbate peroxidase; CAT, catalase; JA, jasmonic acid; MAPK, mitogen-activated protein kinase; NO, nitric oxide; ROS, reactive oxygen species; SA, salicylic acid

subsequent pathogen infection manifested as faster and stronger expression of defense genes compared to naive plants (27). Interestingly, treating plants with BTH induced the accumulation of unphosphorylated, inactive forms of AtMPK3 and 6. Subsequent stress of these primed plants resulted in enhanced accumulation of phosphorylated, active AtMPK3 and AtMPK6, which correlated with enhanced inducibility of *PAL* and *PR-1* expression (7).

MAPK signaling may also play a role either upstream of SA or as part of an SA-positive feedback loop (**Figures 2** and **3**); conditional expression of the MAPK kinase MKK7 induces enhanced resistance to biotrophic pathogens, elevated *PR-1* expression, and the emission of an SAR signal from the *MKK7*-expressing leaves (228). The *ENHANCED DISEASE RESISTANCE 1* (*EDR1*) gene, which encodes a putative MAPKKK, also appears to function upstream of SA; *edr1*-mediated resistance is blocked by expression of the *nahG* transgene or mutations in *NPR1*, *PAD4* or *EDS1* (56). Because *EDR1* encodes a MAPKKK with similarity to CTR1, a negative regulator of ethylene responses, EDR1 may function at the beginning of a MAPK cascade that negatively regulates SA-induced defenses.

AtMPK4, possibly together with its kinase substrates MKS1, WRKY25, and/or WRKY33, represses SA signaling and activates JA signaling (4, 154, 157). AtMPK4 is activated in *Arabidopsis* by *Pseudomonas* infection or by treatment with the *Pseudomonas* PAMP flg22 and may serve to fine-tune AtMPK3- and AtMPK6-mediated defense responses associated with PTI (26, 203). Because *mpk4* displays *EDS1* and *PAD4*-dependent constitutive accumulation of SA and repression of JA signaling, AtMPK4 appears to modulate antagonistic cross talk between SA and JA (14).

Relationship to Cell Death and Reactive Oxygen Species

One of the earliest responses observed after pathogen attack is the OB, which involves a rapid increase in ROS such as superoxide (O_2^-) and H_2O_2 in the apoplast (see sidebar, Accumulation of Reactive Oxygen Species During the Oxidative Burst) (37, 70, 107, 134, 148). The initial increase occurs in response to both

PAMP: pathogen-associated molecular pattern

ACCUMULATION OF REACTIVE OXYGEN SPECIES DURING THE OXIDATIVE BURST

Studies in various plant species suggest that a membrane-bound NADPH oxidase homologous to that activated in human neutrophils ($gp91^{phox}$) generates a rapid increase in apoplastic O_2^- after elicitor or pathogen treatment; O_2^- is then dismutated to H_2O_2 by superoxide dismutase (reviewed in 37). However, H_2O_2 production in elicitor-treated tobacco and bean cells and pathogen-inoculated lettuce appears to be mediated by extracellular peroxidases. ROS generated by chloroplasts and mitochondria also appear to play an important role in the OB (55, 70, 85, 107, 131).

virulent and avirulent pathogens, and can be detected within minutes of infection. Several hours later, a second, sustained burst is detected in plants resistant to the infecting pathogen.

The increased ROS associated with the OB may contribute to resistance via several mechanisms, including directly killing the invading pathogen and/or activating cell wall cross-linking and lignification, thereby strengthening the cell wall and helping confine the pathogen to the infection site (for reviews on SA, ROS, and cell death see 37, 45). In addition, H_2O_2 from the OB was proposed to orchestrate HR-associated defense responses, with high levels activating cell death and low levels signaling defense gene expression. Constitutively elevated ROS also appear to confer enhanced disease resistance based on analyses of transgenic tobacco and potato. Furthermore, SAR development in *P. syringae*-infected *Arabidopsis* may involve the formation of microbursts, in which small aggregates of cells in the systemic leaves accumulate H_2O_2.

The relationship between SA and ROS is complicated. This has led to contradictory results and considerable controversy since the discovery of the first SABP, catalase, and its inhibition by SA. To reconcile a considerable body of conflicting results concerning whether SA is upstream of ROS or vice versa, several researchers proposed that SA and H_2O_2 form a self-amplifying feedback loop (**Figure 3**).

In addition to regulating H_2O_2 production, low concentrations of SA potentiate cell death in pathogen-treated soybean suspension cells. Further linking SA and cell death, HR development in TMV-infected tobacco is delayed when SA accumulation is blocked at early, but not later, times after infection. Additionally, *nabG* expression blocks spontaneous lesion formation in several *Arabidopsis* mutants, including *acd5*, *acd6*, *acd11*, *ssi1*, *cpr22*, *lsd6*, and *lsd7*; lesion formation was generally, although not always, restored by SA, INA, or BTH treatment (106). *sid1/eds5* or *sid2/eds16* also suppressed HR development in pathogen-infected wt or *AtMYB30*-overexpressing *Arabidopsis* (142, 158), and reduced runaway cell death in *lsd1*, *acd11*, *acd6-1*, or *syp121/122* (13, 108, 195, 230).

The interrelationship between SA, cell death, and H_2O_2 led to the hypothesis that defense responses are regulated via an oxidative cell death loop. In this model, the initial H_2O_2 increase following pathogen infection activates SA synthesis; increased levels of SA then work with ROS generated during the second, sustained phase of the OB to potentiate cell death and defense gene expression. SA also potentiates H_2O_2 production, which in turn activates the synthesis of more SA and cell death in a self-amplifying loop (reviewed in 148). Recent studies suggest that ROS levels and cell death also are regulated via mitochondria and chloroplasts (reviewed in 55, 70, 85, 107, 134). In tobacco suspension cells, treatment with SA, H_2O_2, or antimycin A, a specific inhibitor of mitochondrial function, suppressed respiration, increased intracellular ROS, and induced several genes that are induced during programmed cell death (121, 145, 217). Chloroplast-generated H_2O_2 production also is regulated by SA (118, 119), and the redox status of the plastoquinone pool was recently shown to regulate cellular ROS homeostasis and cell death in *Arabidopsis* exposed to excess light (131) (see Constitutive Defense Response Mutants). An additional link between chloroplasts and cell death comes from the combined observations that HR development is light

dependent in several plant-pathogen systems and that SA accumulation and HR intensity increase in proportion to day length after infection (67 and references therein).

Although many studies implicate SA in signaling cell death, there are some exceptions. For example, *nahG* expression failed to suppress spontaneous lesion formation in some *lsd* mutants, and *sid1* and *sid2* developed an HR following inoculation with a high titer of *P. syringae* (37, 106, 142). Differences in the phenotypes of *acd11/nahG*, *acd11/eds5-1*, and *acd11/sid2-2*, combined with the differing ability of SA to restore HR development in these plants, led Brodersen and coworkers (13) to conclude that additional signals work with SA to promote cell death. By contrast, SA appears to negatively regulate cell death in *agd2*, *hrl1*, and *acd6* because a pathogen-induced HR was restored by *nahG* expression (41, 162, 163). However, because pretreating wt *Arabidopsis* with SA or BTH also suppressed HR development, an alternative explanation is that constitutively activated SAR negatively regulates cell death (41).

Nitric Oxide–Salicylic Acid Feedback Loop

Another signaling molecule that interacts with SA is nitric oxide (NO). NO is involved in multiple regulatory processes in mammals and plants (reviewed in 68, 132, 211). In plants, NO and SA appear to function in a positive feedback loop (**Figure 3**); NO donors induce SA accumulation, and NO signaling in defense requires SA (68, 211). Supporting this hypothesis, *nahG* expression suppressed NO-inducible local and systemic resistance in TMV-infected tobacco, whereas SA-induced SAR was compromised by an NO scavenger or inhibitors of NO synthesis (183). It is unclear how NO exerts its functions, but one possible mechanism is through S-nitrosylation of proteins (68), including NPR1 (192); the involvement of S-nitrosylation in SAR has been demonstrated (168) and may act through NPR1. Moreover, NO is involved locally in the induction of cell death (HR) in conjunction with SA, H_2O_2, and ethylene (68, 132, 135, 211).

NO: nitric oxide

Cross Talk Between Salicylic Acid–Jasmonic Acid

Studies over the past ten years have revealed extensive cross talk between hormonal signaling pathways (reviewed in 12, 165). SA generally antagonizes JA signaling by processes that are (partially) dependent on NPR1, SSI2, WRKY transcription factors, and MPK4, although synergism between both signaling pathways has been observed (see above and 12, 92, 133, 186). SA and JA control resistance against pathogens with different infection strategies; SA signaling predominantly combats biotrophic pathogens and viruses, whereas JA signaling protects against necrotrophic pathogens and insects (61). Because one pathway represses the other, plants appear to trade off the costs and benefits of different defense responses (12).

Although antagonism between SA and JA is bidirectional, the main flow of regulation appears to be repression of JA signaling via SA-dependent cues (**Figure 3**). Microarray studies using different SA- and JA-signaling mutants identified considerably more JA-dependent genes repressed by SA signaling than SA-dependent genes repressed by JA signaling (62). Moreover, competition experiments using biotrophic and necrotrophic pathogens or insects revealed a prioritization of SA over the JA pathway in *Arabidopsis* (91). Environmental factors, as well as the types of pathogens infecting plants and the concentrations of SA and JA relative to each other, can affect the outcome of the resulting cross talk (see below and 133, 186). For example, JA-marker gene expression and resistance to the necrotrophic pathogen *Alternaria brassicicola* were downregulated by preinfection of *Arabidopsis* with virulent *P. syringae*, but not by preinfection with avirulent strains of the same pathogen that induce a faster, stronger SA response accompanied by an HR (186, 187).

SA-JA cross talk appears to be at least partially regulated by (transient) changes in the

cellular redox status (**Figure 3**). SA antagonism of JA signaling was abolished by a glutathione biosynthesis inhibitor (91). Furthermore, glutaredoxin 480 (GRX480) represses *PDF1.2* expression in *Arabidopsis*. Expression of *GRX480* is induced by SA in an *NPR1*-dependent manner, and GRX480 interacts with TGA factors in vivo. Whereas overexpression of *GRX480* slightly reduced induction of *PR-1* by SA, it virtually abolished induction of *PDF1.2* by MeJA in an *NPR1*-independent, *TGA2/5/6*-dependent manner (143).

Cross Talk Between Salicylic Acid and Other Hormones

Various hormones involved in plant development communicate with SA. For example, gibberellic acid may affect disease resistance by modulating the SA-JA equilibrium (139, 165). However, SA cross talk with other hormones can be more direct (reviewed in 57, 165). The growth hormone auxin enhances pathogen susceptibility (19, 140); SA was recently shown to repress auxin signaling, thereby reducing susceptibility (**Figure 3**) (207). The finding that enzymes involved in auxin amino acid conjugation, and thus inactivation, affect SA-mediated defenses provides another possible level of cross talk between SA and auxin. GH3.5 conjugates both SA and indole acetic acid, and altered expression of this enzyme affects disease resistance (discussed in Salicylic Acid Metabolism),

Modes of cross talk between abscisic acid (ABA), involved in adaptation of plants to abiotic stress, and SA and JA signaling, as well as ABA's role in biotic stress adaptation, have been extensively reviewed elsewhere (57, 120, 165). ABA may indirectly affect SA signaling via its effect on JA signaling (**Figure 3**) (2) and/or vice versa. Cross talk between SA and ABA is bidirectional (**Figure 3**); whereas an active SA analog repressed NaCl-induced expression of ABA biosynthesis and response genes, ABA or NaCl treatment of *Arabidopsis* antagonized BTH-induced resistance (223). In the latter case, ABA-dependent repression of BTH-induced resistance and *PR-1* gene expression was independent of JA signaling. Yasuda et al. (223) did not observe an effect of ABA treatment on PTI to *P. syringae*. By contrast, de Torres-Zabala et al. (30) showed that ABA signaling positively correlated with susceptibility to *P. syringae* carrying a functional type III secretion system, and that at least one *Pseudomonas* effector protein enhanced ABA accumulation, presumably enhancing virulence of the bacteria. ABA also may antagonize ETI because the constitutive resistance phenotype of *snc1* is suppressed by the *enhanced response to abscisic acid 1* mutation (63).

CONCLUDING REMARKS

Much has been learned during the past two decades regarding how the SA signal is generated, regulated, and transduced to result in HR cell death, defense gene expression, and/or SAR (summarized in **Figures 1** to **3**). One of the biggest outstanding questions in SA biology today concerns how this molecule is initially perceived and how this event triggers resistance signaling. Four SA-binding proteins have been characterized, yet none is the SA receptor. Efforts to elucidate early signaling events after SA induction have been hampered by the various feedback loops that modulate (usually strengthen) the SA signal. Therefore, a major challenge will be to resolve early recognition and signaling events before they are obscured by signal potentiation.

Although it is well established that SA accumulation is required in systemic tissue of locally infected plants to induce SAR, many components of the SAR signal and how they cooperate with SA to induce systemic resistance are still unknown or relatively poorly characterized (203). SAR transcriptionally mimics a local basal defense (PTI) response (114), which is a slower, less intense form of ETI (193). A challenge will be to determine which signal(s) are mobile and minimally required to initiate the systemic SA-potentiating loop that induces SAR.

Finally, SA negatively and positively interacts with several other plant hormones and

signaling molecules that not only affect defense but also regulate developmental processes. An ongoing challenge is to unravel how these interactions affect different processes that are occurring in parallel. Does SA-mediated defense to one pathogen compromise resistance to another? To what extent does the developmental state of a plant affect its capacity to induce SA-mediated defense and vice versa? Answers to these questions should unveil subtle mechanisms used by plants to maximize yield by balancing development and stress resistance.

SUMMARY POINTS

1. In addition to minor roles in the regulation of flowering and thermogenesis, SA plays a major role(s) in basal resistance (PTI), ETI, and SAR.
2. Although accumulation of SA is required in systemic tissues to establish SAR, part of the mobile defense signal for SAR is not SA, but the SA derivative MeSA.
3. The SA defense signal is potentiated by positive feedback loops of SA with NO, ROS, and the *EDS1* and *PAD4* gene products.
4. Signaling downstream from SA is regulated by *NPR1*-dependent and -independent processes. Signal transduction via NPR1 is coregulated by redox changes affecting subcellular localization of NPR1 as well as its interaction with TGA transcription factors.
5. SA induces cell death in cooperation with ROS and NO.
6. The SA signal interacts with other hormone signaling pathways. SA primarily antagonizes JA as well as ABA and auxin signaling, whereas JA and ABA in turn repress SA signaling.

FUTURE ISSUES

1. How is SA recognized and/or what is the receptor for SA, or does SA primarily act through a variety of effector proteins whose activities are altered by SA binding?
2. What is the composition of the mobile SAR signal? Do specific SAR signals exist, or is the mobile signal a copy of the basal defense signature?
3. To what extent does SA affect subcellular localization of proteins, and which proteins are subject to, for instance, nucleo-cytoplasmic trafficking? In that light, how important is nucleo-cytoplasmic trafficking of proteins for SA signaling?
4. To what extent is SA signaling regulated via protein modification, including ubiquitination and sumoylation? And what are the target proteins and their functions?
5. What is the mechanism through which ROS generated in the apoplast, chloroplast and/or mitochondria work with SA to induce HR development and defense gene expression?
6. What is the structure of the hormonal cross-talking network(s), and to what extent are developmental processes affected by pathogen attack and the concomitant induction of defenses, and vice versa?
7. How can we use our knowledge of SA biology to maximize both stress resistance and crop yield?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank our colleagues for providing recent reprints and preprints of their studies. We apologize to those whose work was not covered in this review and/or was only referenced indirectly through other reviews owing to space limitations. We thank Drs. Imre Somssich and Jane E. Parker for critical reading of the manuscript. A.C. Vlot was supported by an EU Marie Curie fellowship (MEIF-CT-2006-040357). The SA studies carried out by the authors were supported by grants to D.F. Klessig from the National Science Foundation including IOS-0525360.

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NOTE ADDED IN PROOF

During the proofreading stage of this review, Attaran et al. published a study showing that two independent *Arabidopsis* knockout mutants of *BSMT1* accumulated severely reduced amounts of MeSA in response to pathogen infection, yet were fully capable of developing SAR. The authors suggest that MeSA as an SAR signal is not conserved between tobacco and *Arabidopsis* (1). In contrast, our analyses of another knockout mutant of *BSMT1* showed that it both failed to accumulate MeSA following infection and was compromised for SAR but not for PTI or ETI (2).

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Annual Review of Phytopathology

Volume 47, 2009

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Errata

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