CURRENT REVIEW

Systemic Acquired Resistance and Salicylic Acid: Past, Present, and Future

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Accepted 16 May 2018.

Salicylic acid (SA) is a critical plant hormone that regulates numerous aspects of plant growth and development as well as the activation of defenses against biotic and abiotic stress. Here, we present a historical overview of the progress that has been made to date in elucidating the role of SA in signaling plant immune responses. The ability of plants to develop acquired immunity after pathogen infection was first proposed in 1933. However, most of our knowledge about plant immune signaling was generated over the last three decades, following the discovery that SA is an endogenous defense signal. During this timeframe, researchers have identified i) two pathways through which SA can be synthesized, ii) numerous proteins that regulate SA synthesis and metabolism, and iii) some of the signaling components that function downstream of SA, including a large number of SA targets or receptors. In addition, it has become increasingly evident that SA does not signal immune responses by itself but, rather, as part of an intricate network that involves many other plant hormones. Future efforts to develop a comprehensive understanding of SA-mediated immune signaling will therefore need to close knowledge gaps that exist within the SA pathway itself as well as clarify how crosstalk among the different hormone signaling pathways leads to an immune response that is both robust and optimized for maximal efficacy, depending on the identity of the attacking pathogen.

IDENTIFICATION OF SYSTEMIC ACQUIRED RESISTANCE (SAR) IN PLANTS

By 1933, the ability of animals to develop acquired immunity, manifested as complete or partial immunity to reinfection following recovery from an initial infection, was well documented. *That year, after reviewing the available literature, Kenneth Chester (1933) concluded that plants also developed increased resistance to reinfection and that this acquired immunity likely played an important role in controlling disease outbreaks in nature. Nearly 30 years later, Ross (1961) investigated this phenomenon in tobacco (Nicotiana tabacum),*

using the cultivar Samsun NN. Due to the presence of the *N* gene, which was introgressed from *Nicotiana glutinosa*, Samsun NN plants responded to *Tobacco mosaic virus* (TMV) infection by developing necrotic lesions (termed a hypersensitive response [HR]) in which viral multiplication was restricted. Frank Ross discovered that when Samsun NN plants received a TMV inoculation on three lower leaves, followed 7 days later by viral challenge of two upper, uninoculated leaves, the lesions formed after the secondary infection were much smaller than those formed after a primary (initial) inoculation of upper leaves on control plants (Fig. 1). Since lesions produced on the upper leaves of the control and test plants appeared at the same time but those on the control plants grew much more rapidly, Frank Ross concluded that the enhanced resistance displayed in the test plants affected either viral multiplication, cell-to-cell movement, or both rather than establishment of infection. He termed this phenomenon SAR.

Through further analyses, Frank Ross determined that SAR developed quickly and was long-lasting. Increased resistance in upper leaves (based on decreased lesion size) was detected by 2 days after the primary inoculation, a maximal level of resistance was observed by 7 to 10 days postinoculation (dpi), and resistance persisted through 20 dpi. Frank Ross also noted that SAR could not be induced by chemical injury, mechanical damage, or inoculation with UV-inactivated TMV or non-lesion inducing viruses. However, it was activated by infection with a variety of local lesion-inducing viruses. Strikingly, SAR conferred heightened resistance to viruses other than that used for the primary inoculation, indicating that it is not specific. This latter observation led Frank Ross to conclude that SAR is not mediated by antibodies but, instead, by a diffusible by-product of infection. He further posited that this factor moves through the veinal system, since cutting the midvein of an upper leaf on the day of the primary infection influenced lesion size after the secondary infection. Uniformly tiny lesions developed basal to the cut, whereas the lesions above the cut ranged from small to large as the apical distance increased. Frank Ross also found that certain environmental conditions influenced SAR, as it was suppressed when test plants were subjected to high temperature (29°C) after the challenge inoculation and was enhanced when plants were provided with continuous light between the primary and challenge inoculations.

Over the following years, researchers determined that TMV infection of resistant tobacco varieties led to the accumulation of several proteins in the inoculated leaves and, subsequently, the upper, uninoculated leaves (Gianinazzi and Kassanis 1974;

Funding: The work summarized here, which was carried out by the authors, was funded by the United States National Science Foundation grant number IOS-0820405.

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Van Loon and Van Kammen 1970). Since these proteins were not detected in i) uninoculated plants, ii) a TMV-infected susceptible variety, or iii) resistant varieties maintained at 30°C after TMV infection (Van Loon 1975), they were proposed to play a role in the resistance process. Additionally, the correlation between the accumulation of these proteins, later named PATHOGENESIS-RELATED (PR) proteins, and the development of resistance in both the inoculated and upper, uninoculated leaves allowed them to serve as effective molecular markers for both local resistance and SAR.

Given that interferon plays an important role in antiviral defense in certain vertebrates, Gianinazzi and Kassanis (1974) tested whether the interferon-inducing compound polyacrylic acid might induce viral resistance and PR protein accumulation in plants. Polyacrylic acid treatment did induce TMV resistance and PR protein accumulation in one TMV-resistant variety of tobacco (Gianinazzi and Kassanis 1974) but not in two others (White 1979). **Instead, Raymond White discovered that TMV resistance and PR protein accumulation could be induced in all three varieties by treatment with benzoic acid (BA),**

salicylic acid (SA), or the SA derivative acetylsalicylic acid (ASA), better known as aspirin (White 1979).

HISTORY OF SALICYLATES AND THEIR MEDICINAL USE

It is alleged that White's mother suggested testing the effect of aspirin on TMV infection because she took aspirin to treat her own maladies. Indeed, SA and its derivatives, collectively known as salicylates, have been used for medicinal purposes for millennia. Long before salicylates were identified, plants rich in salicylates, such as willow, meadowsweet, and poplar, were used for their therapeutic effects. Analyses of the dental plaque from El Sidrón Neanderthals suggest that one individual was chewing poplar bark to relieve the pain of a dental abscess (Weyrich et al. 2017). In the fourth century BC, Hippocrates reportedly encouraged women to chew willow leaves to relieve the pain of childbirth (Vlot et al. 2009). Other ancient cultures that used the bark and leaves of salicylate-rich plants for medicinal purposes include the Assyrians, Babylonians, Romans, Chinese, and the indigenous peoples of the New World (Khan et al. 2015; Pierpoint 1997).

The medicinal properties of willow bark were first studied clinically by the Reverend Edward Stone in the mid-1700s (Pierpoint 1997). The active ingredient in willow bark was purified by Johann Buchner in 1828; he named the yellow, bitter-tasting crystals salicin (Weissmann 1991). Ten years later, Raffaele Piria discovered that salicin could be split into a sugar and an aromatic compound that he named SA, based on the Latin name for white willow, *Salix alba* (Klessig et al. 2016). Analyses of meadowsweet conducted around this time revealed that it contains both salicin and methyl salicylate (MeSA), also known as oil of wintergreen. These compounds are designated 'prodrugs', as they are converted to SA after digestion in humans and animals. To meet the increasing demand for SA, Hermann Kolbe and coworkers developed a chemical process for synthesizing SA in 1859; the first factory for synthetic salicylate production was opened in 1874 (Weissmann 1991). With increased availability and decreased cost, SA use expanded rapidly. However, taking SA at high doses or over long periods was associated with negative side effects, such as stomach irritation and bleeding. Efforts to identify a SA derivative that was better tolerated yet retained the medicinal qualities of SA led to the discovery of ASA by Felix Hoffmann. In 1898, Bayer and Company began synthesizing ASA, using the trade name aspirin, which was created by combining the 'a' from acetyl and 'spirin' from spirsäure, the German name originally given to SA purified from meadowsweet (*Spirea ulmaria*) (Weissmann 1991). Today, aspirin is among the most widely used medications in the world, with an estimated annual consumption of 100 billion tablets. It has been dubbed a wonder drug due to its ability to treat fever, swelling, pain, inflammation, and various skin conditions. Additionally, its prophylactic use reduces the risk of heart attack, stroke, and certain cancers.

RISE OF SA FROM SECONDARY METABOLITE TO PLANT HORMONE

Despite the well-documented therapeutic effects of SA in animals, its function in plants was not uncovered until the late 20th century. SA is one of thousands of phenolic compounds, which consist of an aromatic ring bearing one or more hydroxyl substituents, that are synthesized by plants (Métraux and Raskin 1993). Traditionally, phenolics were assumed to be relatively unimportant or even waste products and, thus, were categorized as secondary metabolites (Raskin 1992). However, subsequent

Fig. 1. Systemic acquired resistance (SAR) in the upper leaves of Samsun NN tobacco plants. **A**, Three lower leaves of Samsun NN plants received either no treatment (control plant on right) or inoculation with *Tobacco mosaic virus* (TMV) (test plant on left). Seven days later, two upper leaves of control and test plants were inoculated with TMV. Plants were photographed 7 days later. **B**, The upper leaf from a test plant (left) and a control plant (right), photographed 7 days after the challenge inoculation. Note the characteristic reduction in lesion size exhibited by SAR-induced test plants as compared with the lesions formed after the primary/initial inoculation of control plants. Reproduced, by permission, from Ross, A. F. 1961 *Virology* 14:340-358. Copyright Elsevier.

studies revealed that plant phenolics have many important functions. Some play critical roles in defense against abiotic stresses, whereas others are important cell-wall structural components, pigments, allelopathic compounds, or signals that influence plant-microbe interactions (Dempsey et al. 2011; Métraux and Raskin 1993; Raskin 1992). In addition, some phenolics play key roles in resisting pest or microbial attack, or both, by serving as constitutively produced antibiotic compounds called phytoanticipins (VanEtten et al. 1994), inducible antimicrobial compounds known as phytoalexins, or signals that activate antimicrobial defense responses (Pieterse et al. 2009; Raskin 1992; Seyfferth and Tsuda 2014; Vlot et al. 2009).

Efforts to identify the function of SA have revealed that it affects a wide range of plant processes. In addition to influencing tolerance to various abiotic stresses (chilling, heat, drought, heavy metal, UV radiation, salinity, or osmotic stress) and inducing resistance to biotic (pathogen-associated) stress, exogenously supplied SA affects numerous aspects of plant growth and development, including seed germination, vegetative growth, root initiation and growth, flowering, fruit yield, senescence, stomatal closure, thermogenesis (heat production), photosynthesis, respiration, the alternative respiratory pathway, glycolysis, and the Krebs cycle (Hayat et al. 2010; Khan et al. 2015; Malamy and Klessig 1992; Miura and Tada 2014; Rivas-San Vicente and Plasencia 2011). SA regulates some of these processes in a concentration-dependent manner, as they are generally induced by treatment with low concentrations of SA and inhibited by high concentrations. This phenomenon may be due, at least in part, to SA-induced changes in the cellular redox status, as low SA concentrations stimulate low-level accumulation of reactive oxygen species (ROS) that can serve as secondary signals for activating biological processes, whereas high SA concentrations induce high-level ROS accumulation, which causes oxidative stress and cell death (Miura and Tada 2014). The mechanisms by which SA regulates many of these processes are not well-characterized. However, they likely involve an array of proteins that bind SA with varying affinities and, as a result, exhibit altered activities that correlate with the SA concentration. In addition, the concerted action of SA and other plant hormones is likely involved (discussed below).

The first suggestion that SA plays an endogenous signaling role in plants was made by Charles Cleland and coworkers. Based on their identification of SA as a phloem-mobile substance that is i) present in vegetative and flowering *Xanthium strumarium* and ii) capable of inducing flowering in *Lemna gibba*, they proposed that SA is a signal for flowering (Cleland 1974; Cleland and Ajami 1974). However, the role of SA in this process remains unresolved. No difference in SA levels was detected in flowering and vegetative *Xanthium strumarium* and, while exogenous SA induces flowering in some plant species, it does not do so in others (Klessig and Malamy 1994; Raskin 1992). Furthermore, while the early or delayed flowering phenotype exhibited by various *Arabidopsis* mutants often correlates with the presence of elevated or reduced SA levels, respectively, exceptions have been noted (Fortuna et al. 2015; Jin et al. 2008; Li et al. 2012; Martínez et al. 2004; Liu et al. 2014; Villajuana-Bonequi et al. 2014; Wang et al. 2011a).

Definitive evidence that SA is a plant hormone first came from efforts to identify the factor regulating thermogenesis in voodoo lily (*Sauromatum guttatum* Schott) (Raskin et al. 1989). During blooming, the spadix (floral spike of the inflorescence) exhibits two episodes of thermogenesis that increase the average surface temperature over 10°C, thereby volatilizing compounds that attract and stimulate insect pollinators. An approximately 100-fold increase in endogenous SA levels was detected prior to each thermogenic event. Thermogenesis also

was induced by treating spadix explants with exogenous SA or its derivatives ASA or 2,6-dihydroxy BA but not by 31 structurally related compounds, arguing that this effect is highly specific. SA was shown to stimulate thermogenesis in the spadix primarily by increasing the capacity of the mitochondrial alternative respiratory pathway (Rhoads and McIntosh 1992). In comparison with the cytochrome pathway, electron flow through the alternative respiratory pathway generates ATP at only one site and the remaining potential energy is released as heat. SA-induced accumulation of alternative oxidase, the terminal electron acceptor in the alternative respiratory pathway, appears to be responsible for the stimulation of this pathway. Interestingly, exogenous SA also induces one or both the alternative respiratory pathway and expression of alternative oxidase in nonthermogenic plant species (Clifton et al. 2005; Norman et al. 2004).

A year after the role played by SA in signaling thermogenesis was first documented, two reports revealed that SA also regulates another plant process, disease resistance.

ROLE OF SA IN PLANT DISEASE RESISTANCE

To ward off infection by the wide array of pathogens found in nature, plants rely on multiple layers of defenses (Asai and Shirasu 2015; Chisholm et al. 2006; Jones and Dangl 2006; Pieterse et al. 2009; Spoel and Dong 2012; Thomma et al. 2011). The first line of defense is comprised of preformed physical barriers, including the cuticle and cell wall, and constitutively produced antimicrobial metabolites. If these passive defenses are breached, active defenses mediated by the plant innate immune system are deployed. One layer of this system is regulated by pattern recognition receptors (PRRs), which are located in the plasma membrane and contain an extracellular domain for binding the foreign ligand. PRRs recognize molecules containing pathogen- or microbe-specific patterns (PAMPs/MAMPs) that are broadly conserved in and unique to microbes. Detection of PAMPs/MAMPs activates pattern-triggered immunity (PTI). PTI often is sufficient to halt further pathogen ingress. However, some pathogens deploy effector proteins capable of suppressing PTI. In these interactions, the plant displays a low level of resistance termed basal resistance. To combat these virulent pathogens, plants utilize the second layer of the innate immune system, known as effector-triggered immunity (ETI) (also called resistance [R] gene-mediated resistance). ETI is mediated by plant-encoded R proteins, which are generally located within the cell. The structure of most R proteins consists of nucleotide-binding and leucine-rich repeat (NB-LRR) domains downstream of an amino-terminal coiled-coil (CC) or toll, interleukin-1 receptor-like (TIR) domain. The interaction of a given R protein with its cognate pathogen-encoded effector protein (also termed avirulence factor) or the perception of a pathogen effector-triggered perturbation of a host protein, leads to induction of ETI.

PTI and ETI are both associated with the activation of various responses in the primary infected tissue. These include the generation of ROS, increased levels of intracellular Ca^{2+} , activation of mitogen-activated protein kinases (MAPKs), synthesis of phytoalexins, extensive transcriptional reprogramming (including increased expression of defense-associated PR genes), and the accumulation of free SA and its 2-O- β -D-glucoside SAG (Pieterse et al. 2009; Seyfferth and Tsuda 2014; Stael et al. 2015; Vlot et al. 2009). These responses are usually induced more rapidly and with greater intensity during ETI than PTI. In addition, ETI is frequently associated with HR development in the inoculated tissue. Several days to a week after these events (depending on the plant-pathogen system), PTI and ETI can induce defense responses in the upper, uninoculated portions of the plant, including the accumulation of PR proteins and SA,

and the development of SAR (Mishina and Zeier 2007). SAR-associated priming of defense responses promotes the faster and stronger response to a secondary infection that is another hallmark of SAR (Conrath et al. 2015). Since SAR was first described by Frank Ross, this long-lasting, broad-based resistance has been observed in a wide variety of plant species following primary infection by viral, bacterial, and fungal pathogens (Dempsey et al. 1999; Fu and Dong 2013; Malamy and Klessig 1992; Pieterse et al. 2009; Spoel and Dong 2012; Vlot et al. 2009).

Yet another layer of defense in the plant arsenal is provided by nonhost resistance. Unlike innate immunity, in which some plant cultivars are resistant to specific isolates of a particular pathogen, nonhost resistance is characterized by resistance of an entire plant species to all isolates of a microbial species (Mysore and Ryu. 2004; Nürnberger and Lipka 2005). While nonhost resistance is the most common mechanism for protecting plants from microbial invasion, it is poorly understood. In addition to preformed defenses, nonhost resistance appears to involve PTI- and ETI-associated signaling components, hormones, and defense responses. This latter observation, combined with the demonstration that nonhost pathogen infection can induce SAR (Mishina and Zeier 2007), suggests that there is at least some overlap between the signaling pathways for innate immunity and nonhost resistance.

In 1990, landmark studies of tobacco and cucumber responding to pathogen infection provided the first evidence that endogenous SA is a signal for disease resistance. In TMV-resistant tobacco, endogenous SA levels rose over 20-fold in the inoculated leaves and more than fivefold in the systemic leaves. These increases paralleled or preceded the accumulation of *PR* gene transcripts (Malamy et al. 1990). In comparison, no increase in SA levels was observed in the TMV-inoculated or systemic leaves of a susceptible tobacco variety. Analyses of phloem exudates from pathogen-infected cucumber likewise detected an over 10-fold increase in SA levels that preceded SAR development and systemic activation of a defense-associated peroxidase (Métraux et al. 1990; Rasmussen et al. 1991; Smith et al. 1991). Confirmation that SA is a critical defense signaling hormone came from studies of SA-deficient tobacco and *Arabidopsis*. John Ryals and coworkers initially demonstrated that PTI, ETI, and SAR are suppressed by expression of the bacterial *nahG* transgene, which encodes the SA-metabolizing enzyme salicylate hydroxylase (Delaney et al. 1994; Gaffney et al. 1993; Lawton et al. 1995). Subsequent analyses demonstrated that mutations in (or sense suppression of) endogenous components involved in pathogen-induced SA accumulation also confer reduced immunity (Dewdney et al. 2000; Nawrath and Métraux 1999; Nawrath et al. 2002; Pallas et al. 1996; Wildermuth et al. 2001).

Although the role of SA as an immune signal for PTI, basal resistance, ETI, and SAR has been documented in many dicot species, its function is less clear in monocots and in plants that constitutively accumulate high levels of SA, such as rice and potato. However, several lines of evidence suggest that SA also serves as a defense signal in these plants. For example, exogenously supplied SA or its synthetic and functional analogs, including benzothiadiazole *S*-methyl ester (BTH), 2,6-dichloroisonicotinic acid (INA), and probenazole (PBZ), induce *PR* gene expression, disease resistance, or both in various monocots (Takatsui 2014; Vlot et al. 2009) and in potato (Kombrink et al. 1996; Navarre and Mayo 2004). In addition, signaling components or mechanisms involved in PTI, ETI, or SAR activation in dicots have been identified in monocots (Balmer et al. 2013b; De Vleeschauwer et al. 2014; Takatsui 2014; Wang et al. 2017) and in potato (Manosalva et al. 2010). Furthermore, recent studies in rice (De Vleeschauwer et al. 2014; Takatsui 2014), wheat (Wang et al. 2017), maize (Balmer

et al. 2013a; Miranda et al. 2017), and potato (Sánchez et al. 2010) have implicated SA as an immune signal during their interaction with at least certain pathogens.

It should be noted that, in addition to SA, several other plant hormones are involved in signaling defenses against microbial pathogen infection (Caarls et al. 2015; De Vleeschauwer et al. 2014; Denancé et al. 2013; Robert-Seilaniantz et al. 2011; Shigenaga and Argueso 2016; Verma et al. 2016). In general, resistance to biotrophic pathogens, which require living host tissue, is mediated via the SA signaling pathway. By contrast, defense responses to necrotrophic pathogens, which feed on dead tissue, are regulated by a different pathway that relies on the hormones jasmonic acid (JA) and ethylene (ET) (Glazebrook 2005; Pieterse et al. 2012). The JA pathway also mediates defenses against insects and herbivory. The substantial crosstalk that occurs between these pathways will be discussed later in this review.

SA AND THE SAR SIGNALS

To develop SAR, a plant must generate a signal in the pathogen-inoculated tissue that travels (presumably through the vasculature) to the uninoculated portions of the plant, in which it signals defense responses. Radio-tracer studies in tobacco and cucumber initially indicated that some of the SA in systemic leaves was synthesized in the inoculated leaf, raising the possibility that SA was the mobile signal (Mölders et al. 1996; Shulaev et al. 1995). Consistent with this possibility, pathogen-induced SA was shown to move through the apoplast prior to phloem loading in *Arabidopsis* (Lim et al. 2016) and SA was detected in phloem sap from pathogen-infected plants (Lim et al. 2016; Métraux et al. 1990; Mölders et al. 1996; Yalpani et al. 1991). However, analyses of chimeric tobacco generated by grafting combinations of wild type (wt) or SA-deficient rootstocks and scions (the upper half of the plant) revealed that plants containing a wt scion developed SAR even if the rootstock was SA-deficient. By contrast, plants containing a SA-deficient scion failed to develop SAR, regardless of the rootstock (Pallas et al. 1996; Vernooij et al. 1994). **Taken together, the results from these critical studies indicated that SA accumulation is required in the uninoculated tissues to signal SAR, but SA is not likely the critical mobile signal.**

Over the years, efforts to identify the mobile SAR signal have identified several candidates. **The first SAR signal to be identified was the SA derivative MeSA. This finding was rapidly followed by the discovery that other compounds, including i) a nine-carbon dicarboxylic acid azelaic acid (AzA), ii) glycerol-3-phosphate (G3P) or a G3P-dependent factor, iii) the abietane diterpenoid dehydroabietinal (DA), and iv) the lysine (Lys) derivative pipecolic acid (Pip), also are mobile inducers of SAR (Fig. 2).** In addition, SAR signaling mediated by some of these small metabolites appears to depend on one or both the lipid transfer protein (LTP) DEFECTIVE IN INDUCED RESISTANCE 1 (DIR1) and the LTP-like protein AZELAIC ACID INDUCED 1 (AZI1). Since these signals and the complex network through which they interact have been the subject of several recent reviews (Dempsey and Klessig 2012; Shah and Zeier 2013; Shah et al. 2014; Singh et al. 2017), only some of the more recent findings will be summarized here.

Genetic, molecular, and biochemical analyses have led Pradeep Kachroo and coworkers to propose that SAR is activated by parallel pathways mediated by SA and AzA/G3P (Singh et al. 2017; Wendehenne et al. 2014). In their model, pathogen infection leads to the accumulation of SA and nitric oxide (NO), which triggers the accumulation of ROS via an amplification loop (Fig. 3). ROS, in turn, generate AzA from precursor C₁₈ unsaturated fatty acids (FAs). AzA then induces the synthesis

and accumulation of G3P, which travels via the symplast to the phloem and subsequently induces SAR, in conjunction with SA, in the systemic tissue (Lim et al. 2016; Wang et al. 2014; Yu et al. 2013). DIR1 and AZI1, which interact with each other as well as themselves, impact this pathway by forming a positive feedback loop with G3P (Yu et al. 2013). Since pathogen-induced accumulation of AzA and G3P as well as SAR were compromised in *Arabidopsis* mutants defective for synthesis of the galactolipids monogalactosyldiacylglycerol (MGDG) or digalactosyldiacylglycerol (DGDG), it was further hypothesized that AzA is generated via oxidation of C₁₈ unsaturated FAs on MGDG and DGDG lipids, rather than free FAs (Gao et al. 2014). Interestingly, the DGDG-defective mutant *dgd1* but not the MGDG-defective mutant *mgd1* failed to accumulate NO or *PR-1* transcripts after pathogen infection; *dgd1* plants also displayed reduced accumulation of free SA and SAG in pathogen-inoculated leaves and reduced free SA accumulation in the systemic leaves. Thus, DGDG and MGDG appear to have additional, distinct functions that impact different steps of the SAR signaling pathway.

Besides regulating G3P synthesis in the inoculated leaf, AzA, AZI1, and DIR1 may serve other roles in SAR signaling. Supporting this possibility, systemic movement of [¹⁴C]AzA required AZI1 and its paralog EARLY ARABIDOPSIS ALUMINUM INDUCED 1 (EARLI1), whereas DIR1, which is important for AzA-induced resistance (Jung et al. 2009), was not required (Fig. 3) (Cecchini et al. 2015a). Consistent with this finding, recombinant DIR1 from *Arabidopsis* did not bind AzA, G3P, or Pip in vitro (Chanda et al. 2011; Isaacs et al. 2016). Additional evidence suggesting that DIR1 and AZI1 have distinct roles in SAR signaling comes from combined findings that i) DIR1 is required for DA-induced SAR activation, whereas AzA, AZI1, and G3P only enhance DA effectiveness (Chaturvedi et al. 2012), ii) AZI1 but not DIR1 interacts with PLASMODESMATA LOCALIZING PROTEIN 1 (PDL1), a protein that localizes to plasmodesmata and is required for SAR (Lim et al. 2016), and iii) AZI1 and EARLI1 but not DIR1 localize to the outer chloroplast membrane and their presence in chloroplasts is enhanced by pathogen infection (Cecchini et al. 2015a). However, AZI1, EARLI1, or both colocalized with DIR1 at several other locations, including the endoplasmic reticulum, plasma membrane or plasmodesmata, and putative contact points between chloroplasts and the endoplasmic reticulum (Cecchini et al. 2015a; Yu et al. 2013). All three proteins also were shown to interact with one or both themselves and each other, and overexpression of DIR1 or AZI1 restored SAR in the reciprocal *azi1* or *dir1* mutant. Together, these findings suggest that DIR1, AZI1, and EARLI1 have both overlapping and distinct cellular locations and functions. Furthermore, since complexes of these proteins are present at sites that abut chloroplasts (Cecchini et al. 2015a) and chloroplasts are associated with the generation of G3P, DA, AzA, MeSA (via production of SA) (Cecchini et al. 2015a), and, potentially, Pip (Ding et al. 2016a), these proteins may play critical roles in mobilizing various SAR signals from their site of production to the phloem.

Analyses of *Arabidopsis* mutants defective for SA biosynthesis due to mutations in *SA INDUCTION DEFICIENT 2* (*SID2*) (also termed *ICS1* for *ISOCHORISMATE SYNTHASE 1*, discussed below) or downstream SA signaling components have confirmed that full SAR induction by all of the candidate signals requires SA synthesis or accumulation, or both, and a functional SA signaling pathway in the systemic leaves (Fig. 3) (Shah et al. 2014). One possible mechanism for systemic SA accumulation involves MeSA, which is synthesized from SA in pathogen-infected leaves, transported via the phloem to the distal leaves, and then converted back to SA (Dempsey and Klessig 2012; Shah and Zeier 2013; Shah et al. 2014). In addition, systemic SA synthesis appears to be regulated by a positive feedback loop involving Pip, AGD2-LIKE

DEFENSE RESPONSE PROTEIN 1 (*ALD1*), SAR-DEFICIENT 4 (*SARD4*), FLAVIN-DEPENDENT MONOOXYGENASE 1 (*FMO1*), PHYTOALEXIN-DEFICIENT 4 (*PAD4*), *ICS1*, and *SA* (Ding et al. 2016a; Hartmann et al. 2017; Návárová et al. 2012).

Recent studies of the Pip biosynthetic pathway indicate that *ALD1* mediates the first step by converting L-Lys to ε-amino-α-ketocaproic acid (KAC), which can cyclize to form Δ¹-piperidine-2-carboxylic acid (P2C) (also designated 1,2-dehydropipecolic acid [DP]) (Ding et al. 2016a; Hartmann et al. 2017). Pip is then generated by *SARD4*-mediated reduction of 1,2-DP or its isomer, 2,3-DP, while a *SARD4*-independent mechanism appears to play a minor role in Pip synthesis (Fig. 3). Analyses of *sid2* and *ald1* single and double mutants have suggested that pathogen-induced systemic SA accumulation requires *ALD1*, whereas systemic Pip accumulation is substantially reduced, but not abrogated by the loss of *SID2* (Bernsdorff et al. 2016). These studies further indicated that SAR and transcriptional upregulation of most SAR-associated genes in the systemic tissue are largely dependent on SA, although a SA-independent, Pip-dependent pathway also is involved. In the primary inoculated leaves, the SA and Pip pathways were observed to contribute additively to basal resistance, with the SA pathway playing a greater role in this process. Interestingly, petiole exudates from *Arabidopsis* overexpressing *ALD1* enhanced basal resistance in wt and *ald1* plants, although they did not contain elevated levels of Pip or SA (Cecchini et al. 2015b). This finding led to the suggestion that one or more *ALD1*-generated, non-Pip metabolites regulates basal resistance (Cecchini et al. 2015b). Alternatively, the petiole exudates might contain elevated levels of 2,3-DP; this Pip precursor enhanced basal resistance in wt and *ald1* plants (Hartmann et al. 2017). An important clue as to how parallel SA- and Pip-dependent signaling pathways are activated after pathogen infection comes from the discovery that *ICS1*, *ALD1*, and *SARD4* expression as well as SA and Pip accumulation are regulated by the transcription factors (TFs) SAR DEFICIENT 1 (*SARD1*) and CALMODULIN-BINDING PROTEIN 60g (CBP60g), which, in turn, are upregulated by TGACG-BINDING FACTOR 1 (*TGA1*) and *TGA4* (Sun et al. 2017; Wang et al. 2011b; Zhang et al. 2010).

It should be noted that MeSA is a highly volatile compound that, in addition to moving through the vasculature, can travel through the air to induce defense responses in both pathogen-inoculated plants and neighboring, uninfected plants, presumably following its conversion back to SA (Dempsey and Klessig 2012;

Fig. 2. The structure of salicylic acid (SA) and the long-distance systemic acquired resistance signals methyl salicylate (MeSA), azelaic acid (AzA), glycerol-3-phosphate (G3P), dehydroabietinal (DA), and pipercolic acid (Pip).

Dempsey et al. 2011). By comparing the volatile organic compounds emitted during SAR signaling by wt *Arabidopsis* and a SAR-defective mutant, Corina Vlot and coworkers recently identified several closely related monoterpenes as volatile SAR signals that activate defenses both within and between plants (Riedlmeier et al. 2017). Monoterpene-induced resistance was dependent on SA biosynthesis or signaling and *AZI1* but not G3P (Fig. 3). Mutations in GERANYLGERANYL REDUCTASE (GGR), which is involved in monoterpene synthesis, compromised SAR activation not only in the mutant plants but, also, in neighboring, uninfected plants maintained in the same gas-tight desiccator. The ability of wt versus *ggr* plants to propagate plant-to-plant immune signaling correlated with the presence or absence, respectively, of monoterpenes in their emissions.

CLASSICAL AND TRADITIONAL BIOCHEMICAL APPROACHES TO IDENTIFY SA RECEPTORS

To determine the mechanisms through which SA signals plant defense responses, Daniel Klessig and coworkers sought to identify SA-binding proteins (SABPs) using a biochemical

approach (Kumar 2014). *Starting with extracts from tobacco leaves, they isolated the first SABP; it was an abundant, soluble protein that i) reversibly bound SA, ii) displayed a physiologically relevant affinity for SA (dissociation constant [K_d] = 14 μ M), and iii) bound biologically active but not inactive SA analogs (Chen and Klessig 1991; Chen et al. 1993a).* This SABP was subsequently identified as CATALASE (CAT), and its H_2O_2 -scavenging activities were found to be inhibited by binding to SA, biologically active SA analogs, or the functional SA analogs INA and BTH (Chen et al. 1993b; Conrath et al. 1995; Durner and Klessig 1996; Wendehenne et al. 1998). Additional studies revealed that SA, INA, BTH, and biologically active SA analogs also inhibited the activity of ASCORBATE PEROXIDASE (APX), the other major H_2O_2 -scavenging activity in cells (Durner and Klessig 1995; Wendehenne et al. 1998).

Plant cells continuously generate H_2O_2 as a byproduct of cellular metabolism (Baxter et al. 2014). In addition, rapid and transient increases in ROS, including H_2O_2 , occur during immune responses triggered by pathogen infection. Thus, it was proposed that pathogen-induced increases in SA inhibit catalase and APX activity, thereby promoting the accumulation of

H₂O₂ and H₂O₂-derived ROS, which could serve as secondary messengers for activating immune responses (Chen et al. 1993b; Durner and Klessig 1995). Multiple studies assessing catalase and APX activity, ROS levels, and defense activation, singly or in combination, in plants responding to exogenously supplied SA, H₂O₂, or pathogen infection yielded conflicting results (Dempsey et al. 1999). However, the combined observations that i) *sid2-2* plants are compromised for pathogen-induced decreases in catalase activity and increases in H₂O₂ concentration and ii) basal immunity was partially restored when the *cat2-1* mutation was introduced into the *sid2-2* background provide strong support for this hypothesis (Yuan et al. 2017).

The next SABP (SABP2) to be identified was purified from tobacco extracts using very high specific activity radio-labeled SA (Du and Klessig 1997). Analyses of SABP2 indicated that it is a member of the α/β -fold hydrolase family that i) reversibly binds SA with high affinity ($K_d = 90$ nM), ii) plays an essential role in SAR, and iii) exhibits SA-inhibitable MeSA esterase activity (Du and Klessig 1997; Forouhar et al. 2005; Kumar and Klessig 2003). Grafting studies using chimeric tobacco containing wt or *SABP2*-silenced rootstocks or scions, or both, demonstrated that *SABP2* expression in the scion but not the rootstock is necessary for SAR development (Park et al. 2007a). A complementary grafting study revealed that expression of the MeSA-synthesizing enzyme SA METHYL TRANSFERASE 1 (NtSAMT1) is required in the rootstock but not in scions for SAR development. Since MeSA does not appear to be biologically active, the following scenario for SAR signaling was proposed. Some of the accumulating SA in the pathogen-inoculated leaf is converted to MeSA by NtSAMT1; the high levels of SA in this tissue further promote MeSA accumulation by inhibiting the methyl esterase activity of SABP2 (Fig. 3). Following translocation to the systemic tissues via the phloem, MeSA is converted back to SA by SABP2, and the released SA activates or primes systemic immune responses (Dempsey et al. 2011). A similar signaling pathway appears to operate in *Arabidopsis* and potato, since suppressing the expression or activity of their SABP2 orthologs, including multiple members of the

Arabidopsis METHYL ESTERASE (AtMES) family or potato StMES1 or the *Arabidopsis* ortholog of NtSAMT1, BA/SA CARBOXYL METHYLTRANSFERASE 1 (AtBSMT1), compromised one or both systemic SA accumulation and SAR (Liu et al. 2010; Manosalva et al. 2010; Vlot et al. 2008). The extent to which SAR activation relies on MeSA was subsequently found to correlate with the duration of light exposure after inoculation. *Arabidopsis* or tobacco deficient for MeSA synthesis or MeSA esterase activity failed to develop SAR if the inoculation occurred near the beginning of the night, or dark, period but were partially to fully SAR competent if this inoculation was followed by several hours of light (Liu et al. 2011). Interestingly, SAR competence in DIR1 and G3P-deficient *Arabidopsis* mutants also correlated with light exposure after infection (Liu et al. 2011).

To identify additional SABPs, soluble chloroplast proteins from tobacco were assessed for their ability to bind radio-labeled SA. This approach led to the identification of SABP3, which reversibly bound SA with moderate affinity ($K_d = 3.7$ μ M) (Slaymaker et al. 2002). Partial amino acid (aa) sequencing of purified SABP3 revealed that it is a chloroplast CARBONIC ANHYDRASE (CA). Both this protein, designated CA1, and a second CA that is expressed at very low levels, named CA2, bound biologically active but not inactive SA analogs. A major function of CA is to catalyze the reversible hydration of CO₂ to HCO₃⁻ (bicarbonate). In C₄ plants, which accumulate CA in the cytosol, CA-mediated HCO₃⁻ synthesis is the first step in photosynthetic carbon assimilation. By contrast, C₃ plants such as tobacco and *Arabidopsis* accumulate CA primarily in the chloroplast; whether plastidial CA is involved in carbon assimilation is unclear. However, plastidial CA has been implicated in lipid biosynthesis in the C₃ plants cotton and tobacco, potentially by trapping inorganic carbon inside the plastid in the form of HCO₃⁻ (Hoang and Chapman 2002). In addition, complementation analyses in yeast suggested that CA has an antioxidant activity that is independent of its enzymatic activity (Slaymaker et al. 2002).

Although CA was identified as a SABP, SA binding did not affect the enzymatic activity of purified tobacco SABP3

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Fig. 3. A simplified model depicting the long-distance systemic acquired resistance (SAR) signals and the network through which they mediate SAR signaling. Several candidate phloem-mobile SAR signals have been identified, including methyl salicylate (MeSA) (dark blue), dehydroabietinal (DA) (yellow), azelaic acid (Aza) (green), glycerol-3-phosphate (G3P) or a G3P-dependent factor (orange), and pipecolic acid (Pip) (light blue). In addition, MeSA and several closely related monoterpenes (MT) (pink) are highly volatile compounds that can travel through the air to signal SAR in the systemic tissues of the infected plant and in neighboring plants (indicated by wavy lines leaving the inoculated tissue and entering the distal/systemic tissue). In the local, infected tissue, increased expression of *ICS1* (also named *SID2*) leads to salicylic acid (SA) accumulation. A portion of this SA is converted to MeSA by BSMT1 in *Arabidopsis* (or SAMT1 in tobacco). The high SA level in this tissue further promotes MeSA accumulation by inhibiting the MeSA esterase (MSE) activity of certain METHYL ESTERASE (MES) family members in *Arabidopsis* (or SABP2 in tobacco). MeSA travels to uninfected tissue via the phloem (dashed line) or as a volatile, airborne signal (wavy line). Pathogen infection also stimulates the accumulation of Aza, G3P, and Pip in the inoculated tissue. Pip is synthesized from lysine (Lys) by the tandem activities of ALD1 and SARD4. Aza accumulation is proposed to be initiated by the pathogen-induced generation of an unknown signal (designated X) which, in conjunction with digalactosyldiacylglycerol (DGDG), triggers nitric oxide (NO) and SA accumulation. Reactive oxygen species, which form a self-amplification loop with NO, catalyze the formation of Aza via cleavage of C₁₈ unsaturated fatty acids (C₁₈ FAs) potentially bound to monogalactosyldiacylglycerol (MGDG) or DGDG. In planta movement of Aza is dependent on the lipid transfer-like protein AZI1 and its paralog EARLI1. Aza also induces the synthesis of G3P. Accumulation of G3P is further promoted by a positive feedback loop involving AZI1 and DIR1. In comparison with these SAR signals, the absolute level of DA does not change in the pathogen-inoculated tissue. Instead, the presence of DA in a high molecular mass, biologically active complex (indicated as DA*) is enhanced. DA-induced SAR and Aza-induced local resistance are dependent on DIR1, although the role of DIR1 is not understood. Finally, SAR induction is associated with the emission of several closely related monoterpenes (MT). GERANYLGERANYL REDUCTASE (GGR), which is a subunit of the heteromeric enzyme GERANYLDIPHOSPHATE SYNTHASE, is required for MT synthesis. In the distal/systemic tissue, MeSA (arriving via the phloem or air) is converted back to SA via the MSE activity of AtMES or NtSABP2 and the released SA induces or primes systemic defense responses predominantly via an NPR1-dependent pathway. Several SAR signals influence the conversion of MeSA to SA in systemic tissue, as *AtMES9* expression is induced by DA* and G3P, while *AtBSMT1* or *NtSAMT1* expression is suppressed by G3P and DIR1. Whether G3P also functions in parallel with SA in the systemic tissue in addition to acting upstream of SA is currently unclear. Systemic SA accumulation also is regulated by an amplification loop that involves Pip, ALD1, SARD4, FMO1, ICS1, and SA. PAD4 functions in this amplification cycle by promoting the expression of *ALD1*, *FMO1*, and *ICS1*. Additionally, DA* and Aza feed into this cycle, as they require FMO1 to induce SAR. While Pip induces SAR primarily via the SA- and NPR1-dependent pathway, it also induces modest levels of SAR via a SA-independent pathway that may involve NPR1. The mechanism through which MT induces SAR is currently unknown, although it requires AZI1 and a functional SA signaling pathway. Dashed arrows represent phloem-associated movement of SAR signals while wavy lines indicate airborne movement. Colored ovals denote SAR signals, while boxes indicate proteins involved in SAR signaling. Arrows indicate positive regulation, while lines ending in a bar indicate negative regulation. The arrow from SA to SAR via NPR1 is substantially thicker than that associated with the Pip-dependent, SA-independent pathway; this represents the greater importance of SA-dependent signaling in SAR induction.

(Slaymaker et al. 2002). Instead, *S*-nitrosylation, which involves addition of a NO moiety, inhibited both SA binding and the CA activity of AtSABP3, the *Arabidopsis* ortholog of SABP3 (Wang et al. 2009). Following infection of *Arabidopsis* with an avirulent bacterial pathogen but not with a virulent one the level of *S*-nitrosothiol (SNO)-AtSABP3 rose over time, and this correlated with a marked decrease in CA activity. This finding, combined with the observation that i) CA-deficient *Nicotiana benthamiana* and *Arabidopsis* were compromised for PTI/basal resistance or ETI to various pathogens as well as HR development triggered by the Pto:avrPto interaction (Poque et al. 2018; Restrepo et al. 2005; Slaymaker et al. 2002; Wang et al. 2009), ii) *Arabidopsis* overexpressing *AtCA1* displayed comparably enhanced susceptibility to Turnip mosaic virus (TuMV) as the *Atca1* knockout mutant, and iii) the *AtCA1* overexpression and knockout mutants were compromised for TuMV-induced SA accumulation and displayed either no induction or an altered pattern of *PR* gene expression (Poque et al. 2018), suggested that CA is a critical regulator of SA-mediated immunity at early timepoints after infection but its activity must be down-regulated subsequently to avoid antagonizing the SA pathway (Poque et al. 2018; Wang et al. 2009). It was recently demonstrated that the TuMV-encoded protein HELPER-COMPONENT PROTEINASE (HCPPro) not only binds *AtCA1* in the cytoplasm but also negatively regulates *AtCA1* transcript and protein levels (Poque et al. 2018). Since transient expression of TuMV HCPPro in *Arabidopsis* suppressed SA-induced *PR* gene expression, HCPPro may promote viral infection via its ability to suppress *AtCA1* and thus SA-induced defenses.

GENETIC AND MOLECULAR APPROACHES TO ELUCIDATING THE SA SIGNALING PATHWAY

Concurrent with these classical biochemical studies, other researchers were using genetic and molecular approaches, singly or together, to i) elucidate the SA biosynthetic pathway, ii) identify the proteins responsible for regulating SA biosynthesis and metabolism, and iii) identify the SA receptors and downstream components involved in immune signaling.

SA biosynthesis.

The combined results from radio-tracer, sense-suppression, and inhibitor studies initially suggested that plants generate SA via the PHENYLALANINE AMMONIA-LYASE (PAL) pathway (Dempsey et al. 2011; Gao et al. 2015; Khan et al. 2015; Seyfferth and Tsuda 2014). PAL, which is the first enzyme in phenylpropanoid synthesis, converts phenylalanine to *trans*-cinnamic acid (*t*-CA) (Fig. 4). Depending on the plant species, *t*-CA is then converted to SA via the intermediates *ortho*-coumaric acid or BA. SA is presumably generated from BA by BA 2-HYDROXYLASE (BA2H), although this enzyme has not been purified. Modulation of *PAL* expression has been shown to impact pathogen-induced SA accumulation and disease resistance in *Arabidopsis*, pepper, soybean, and tobacco (Huang et al. 2010; Kim and Hwang 2014; Pallas et al. 1996; Shine et al. 2016). However, it should be noted that *Arabidopsis* containing mutations in all four *PAL* genes displayed a 90% reduction in basal PAL activity but only a 50% decrease in pathogen-induced SA accumulation (Huang et al. 2010).

Prior to the analysis of *PAL*-deficient *Arabidopsis*, the existence of a *PAL*-independent SA biosynthetic pathway was suggested by the inability of a *PAL*-specific inhibitor to fully suppress pathogen- or chemical-induced SA accumulation and the occasionally lower-than-expected incorporation of radio-labeled precursors into SA (Wildermuth et al. 2001). It was

known that several genera of bacteria synthesize SA from chorismate, the end-product of the shikimate pathway, via an isochorismate (IC) intermediate (Dempsey et al. 2011; Seyfferth and Tsuda 2014). In some bacterial species, SA is generated from chorismate by a single enzyme, SA SYNTHASE. Other species utilize a two-step pathway in which chorismate is isomerized to IC by ISOCHORISMATE SYNTHASE (ICS) and IC is then converted to SA by ISOCHORISMATE PYRUVATE LYASE (IPL). *Based on the knowledge that prokaryotic endosymbionts gave rise to many plastid pathways and that chorismate is synthesized in chloroplasts, Wildermuth et al. (2001) searched the newly sequenced Arabidopsis genome and, in a landmark discovery, identified two putative ICS genes.* Mutations in *ICS1*, previously identified as *sid2-1* and *sid2-2* (also designated *ENHANCED DISEASE SUSCEPTIBILITY [eds]16-1*), conferred reduced immunity (Dewdney et al. 2000; Nawrath and Métraux 1999; Wildermuth et al. 2001). Furthermore, SA accumulation was reduced by 90 to 95% in these mutants, indicating that the IC pathway is the major route for pathogen-induced SA synthesis in *Arabidopsis* (Fig. 4).

In comparison with *ICS1*, *ICS2* generates only minimal levels of SA after pathogen infection (Garcion et al. 2008). Both *ICS2* and *ICS1* are imported into the chloroplast, and biochemical analyses recently demonstrated that *ICS2* is enzymatically active, with a reaction rate approximately half that of *ICS1* (Macaulay et al. 2017). However, *ICS2*, unlike *ICS1*, is constitutively expressed in veins and hydathodes and is not induced by pathogen infection or SA treatment (Hunter et al. 2013; Macaulay et al. 2017; Wildermuth et al. 2001). Thus, the different expression patterns of these proteins appear to be responsible for their disparate contributions to pathogen-induced SA accumulation.

Additional studies have implicated the IC pathway in pathogen-induced SA accumulation in several plant species and identified many *ICS* homologs (Dempsey et al. 2011). It should be noted, however, that a plant gene corresponding to IPL has yet to be identified (Dempsey and Klessig 2017; Gao et al. 2015; Seyfferth and Tsuda 2014). Interestingly, the IC pathway in soybean appears to work cooperatively with the PAL pathway to generate pathogen-induced SA (Shine et al. 2016).

Regulation of SA synthesis.

Characterization of *Arabidopsis* mutants led to the identification of several proteins that are required for pathogen-induced SA accumulation and immunity. These include the lipase-like proteins ENHANCED DISEASE SUSCEPTIBILITY 1 (*EDS1*) and *PAD4* and the integrin-like protein NONSPECIFIC DISEASE RESISTANCE 1 (*NDR1*) (Dempsey et al. 2011; Fu and Dong 2013; Knepper et al. 2011; Vlot et al. 2009). All three proteins positively regulate basal resistance to biotrophic pathogens. *NDR1* also mediates ETI triggered by many but not all CC-NB-LRR type R proteins, whereas *EDS1* and *PAD4* mediate ETI activated by TIR-NB-LRR R proteins. Additionally, *EDS1* and *PAD4* contribute to resistance signaled by CC-NB-LRR proteins by working redundantly with SA (Cui et al. 2017; Venugopal et al. 2009). It should be noted that *EDS1* and *PAD4* also mediate basal and TIR-NB-LRR-triggered resistance via a SA-independent pathway that works in parallel with the SA pathway and, thus, may safeguard immune activation when SA accumulation is disrupted (Cui et al. 2017).

EDS1 forms homodimers that are localized in the cytoplasm (Feys et al. 2001, 2005). It also directly interacts with *PAD4* and the lipase-like protein SENESCENCE-ASSOCIATED GENE 101 (*SAG101*) to form exclusive nucleo-cytoplasmic or nuclear complexes, respectively, that have both independent

and cooperating functions critical for resistance signaling (Feys et al. 2001, 2005; Wagner et al. 2013). In addition, EDS1 interacts with several TIR-NBS-LRR R proteins, some of their cognate bacterial effectors, and a CC-NBS-LRR R protein in one or both the cytoplasm and nucleus (Bhattacharjee et al. 2011; Heidrich et al. 2011; Huh et al. 2017; Zhu et al. 2011). Analyses of *Arabidopsis* in which EDS1 trafficking to and from the nucleus was altered revealed that the nuclear pool of EDS1 is essential for full basal resistance and ETI to bacterial and oomycete pathogens, whereas the cytoplasmic pool appears to be needed only for promoting efficient immune responses (García et al. 2010). Notably, nuclear EDS1 levels increased rapidly after ETI activation; this preceded or coincided with transcriptional reprogramming, including the upregulation of *ICS1*, *EDS1*, and *PAD4* (García et al. 2010). Since SA promotes *EDS1* and *PAD4* expression and *EDS1* and *PAD4* promote *ICS1* expression and SA accumulation (albeit via an unknown mechanism), this process appears to initiate a positive feedback loop that can further amplify SA accumulation and defense signaling (Dempsey et al. 2011; Wiermer et al. 2005).

Genetic and molecular analyses also have identified several TFs that act as direct positive regulators of *ICS1* (Birkenbihl

et al. 2017; Dempsey and Klessig 2017; Fu and Dong 2013; Seyfferth and Tsuda 2014). CBP60g and its homolog SARD1 exhibit partially redundant functions that are required for pathogen-induced *ICS1* expression and SA accumulation (Wang et al. 2011b; Zhang et al. 2010). Although CBP60g and SARD1 belong to the same clade of the CBP60 family, CBP60g binds the Ca^{2+} -sensing protein CALMODULIN, whereas SARD1 does not. WRKY28, a member of the WRKY TF family, also binds the *ICS1* promoter and activates *ICS1* expression (van Verk et al. 2011). Since Ca^{2+} has been implicated in the activation of WRKY28 as well as CBP60g, these proteins may provide a link between pathogen-induced Ca^{2+} influxes and SA synthesis (Seyfferth and Tsuda 2014). More recently, TEOSINTE BRANCHED 1/CYCLOIDEA/PCF 8 (TCP8) (Wang et al. 2015), NTM-LIKE 9 (Zheng et al. 2015), and CCA1 HIKING EXPEDITION (also known as TCP21) (Lopez et al. 2015; Zheng et al. 2015) were shown to serve as direct positive regulators of *ICS1* during immune responses. Interestingly, TCP8, which functions redundantly with other TCPs to upregulate *ICS1*, was shown to interact with some TCPs and with several *ICS1*-associated TFs, including SARD1, WRKY28, and ANAC019, a member of the *Arabidopsis* NAM/ATAF1,

The IC Pathway

The PAL Pathway

Fig. 4. Two salicylic acid (SA) biosynthetic pathways have been identified in plants: the phenylalanine ammonia-lyase (PAL) pathway and the isochorismate (IC) pathway. AAO = aldehyde oxidase, BA2H = benzoic acid 2-hydroxylase; CM = chorismate mutase; ICS = isochorismate synthase; IPL = isochorismate pyruvate lyase. Question marks indicate enzymes that are presumed to mediate the indicated conversion but have yet to be identified definitively.

ATAF2/CUC2 (NAC) TF family (Wang et al. 2015). These interactions may provide nuanced *ICS1* expression as well as form regulatory nodes that support crosstalk between different signaling pathways. ANAC019 is one of several negative regulators of *ICS1* that have been identified; others include ETHYLENE INSENSITIVE 3 (EIN3), EIN-like 1 (EIL1), and two other NAC family members, ANAC055 and ANAC072 (Fu and Dong 2013; Shigenaga and Argueso 2016). Since these TFs also regulate JA and ET signaling, they may facilitate crosstalk between all three pathways.

It was relatively recently demonstrated that CALCIUM-SENSING RECEPTOR (CAS), a thylakoid-associated Ca^{2+} -binding protein, is required for basal resistance, ETI, and PAMP-induced SA accumulation (Nomura et al. 2012). Consistent with this finding, the expression patterns for several TFs, including WRKY28, CBP60g, and SARD1, were altered in PAMP-treated *cas-1* mutants and transcript accumulation for genes involved in pathogen-induced SA accumulation, including *ICS1* and *PAD4*, was reduced at early timepoints after PAMP treatment. While the molecular function of CAS is currently unclear, these findings suggest that retrograde signals from the chloroplast play a role in regulating the expression of nuclear-encoded genes associated with SA biosynthesis and immunity (Stael et al. 2015).

Regulation of cytosolic SA levels.

Genetic screens have identified several other genes, including *EDS5* (also designated *SID1*), *avrPphB* *SUSCEPTIBLE 3* (*PBS3*) (also named *GRETCHEN HAGEN 3.12*, *HopW1-INTERACTING*, or *GH3-LIKE DEFENSE GENE 1*), and *ENHANCED PSEUDOMONAS SUSCEPTIBILITY 1* (*EPS1*) that are critical for pathogen-induced SA and SAG accumulation and immunity (Dempsey et al. 2011; Fu and Dong 2013; Seyferth and Tsuda 2014). *EDS5* encodes a member of the multidrug and toxin extrusion transporter family (Nawrath et al. 2002). Subsequent studies localized *EDS5* to the chloroplast envelope, where it was proposed to mediate SA export from the chloroplast to the cytosol (Serrano et al. 2013; Yamasaki et al. 2013). Once in the cytosol, free SA can be modified in various ways that generally render it inactive. These modifications are presumed to serve multiple purposes by i) regulating the level of biologically active free SA in the cytoplasm and ii) providing a reservoir of SA that can be either readily accessed, transported, or both in response to stress. Maintaining low basal SA levels in the absence of pathogen infection is important for plant fitness, as constitutive activation of immune responses may drain energy and resources away from plant growth and reproduction; it also can induce spontaneous necrosis (Denancé et al. 2013; Heil and Baldwin 2002).

Following pathogen infection, much of the newly synthesized SA is glucosylated at the hydroxyl group to form SAG. Less frequently, glucosylation occurs at the carboxyl group to produce salicylate glucose ester. SAG is transported to the vacuole, where it presumably functions as an inactive, nontoxic storage form that can be converted back to free SA in response to subsequent pathogen attack (Dempsey et al. 2011; Fu and Dong 2013; Shigenaga and Argueso 2016). SA also can be methylated to produce MeSA. This modification both inactivates SA and increases its membrane permeability and volatility, thereby facilitating long-distance movement of MeSA (Park et al. 2007a). Pathogen infection also stimulates increases in the level of 2,3-dihydroxybenzoic acid (2,3-DHBA) and, to a lesser extent, 2,5-DHBA (Bartsch et al. 2010). The combined observations that i) SA 3-HYDROXYLASE (S3H) (also termed DMR6-LIKE OXYGENASE 1) (Zeilmaker et al. 2015) mediates the conversion of SA to 2,3-DHBA both in vitro and in planta and ii) the *s3h* mutant accumulates elevated levels of SA

and SAG, suggest that S3H-mediated hydroxylation is a mechanism for preventing SA overaccumulation (Zhang et al. 2013). Conjugation of SA to aa is yet another modification strategy. Salicyloyl-L-aspartate (SA-Asp), which is the dominant stable SA-aa conjugate, is thought to be generated by GH3.5 (also termed WESO 1), a member of the acyl adenylase family (Park et al. 2007b; Staswick et al. 2002). It is currently unclear whether the primary role of GH3.5 in planta is to conjugate SA or whether it, instead, conjugates an alternative substrate, such as the potential SA precursor BA, which could impact SA metabolism, or the auxins indole-3-acetic acid (IAA) or phenylacetic acid (Westfall et al. 2016; Zhang et al. 2007). PBS3 is another member of the GH3 family; it does not use SA or IAA as acyl substrates but, instead, conjugates aa to 4-substituted benzoates (Okrent et al. 2009). Since PBS3 appears to function upstream of SA synthesis and accumulation and stress-induced *pbs3* plants contained elevated levels of SA-Asp, it was hypothesized that PBS3 increases cytosolic SA levels by repressing SA-Asp synthesis (Dempsey et al. 2011). Another metabolic enzyme that influences SA accumulation is EPS1 (Zheng et al. 2009). Although the function of this putative BAHD acyl transferase is currently unclear, EPS1, like PBS3, appears to function upstream of SA, possibly by generating a precursor or regulatory molecule for SA synthesis.

Signaling components downstream of SA.

Following the discovery that SA is an endogenous signal for immunity, several laboratories initiated genetic screens to identify downstream signaling components. *These efforts opened a new chapter in our understanding of SA molecular signaling mechanisms with the identification of a single gene, NONEXPRESSER OF PR GENES 1 (NPR1), also named NON-INDUCIBLE IMMUNITY 1 (NIM1) or SA INSENSITIVE 1 (Cao et al. 1994; Delaney et al. 1995; Glazebrook et al. 1996; Shah et al. 1997).* Characterization of *npr1* mutants revealed that they were compromised for SA- and pathogen-induced immunity, despite accumulating elevated levels of SA. Structural analyses of NPR1 indicated that it contains two domains involved in protein-protein interactions, an N-terminal BTB/POZ (broad-complex, tamtrack, bric-a-brac/pox virus, and zinc finger) domain, and an ankyrin repeat domain as well as a C-terminal transactivation domain and nuclear localization sequence (Gao et al. 2015; Herrera-Vásquez et al. 2015; Kuai et al. 2015; Pajeroska-Mukhtar et al. 2013; Seyferth and Tsuda 2014). Five additional members of the NPR gene family have been identified in *Arabidopsis*; all encode proteins containing the BTB/POZ and ankyrin repeat domains. Strikingly, NPR1 shares significant homology at the aa level with the mammalian immune regulator I κ B (NF- κ B inhibitor) (Ryals et al. 1997).

In uninfected plants, NPR1 predominantly resides in the cytosol in oligomeric complexes formed by intermolecular disulfide bonds (Mou et al. 2003). Cytosolic NPR1 is unable to promote SA-induced defense gene expression. However, it does modulate crosstalk between the SA and JA signaling pathways (Spoel et al. 2003). Following pathogen infection, SA induces a biphasic change in the cellular redox state, in which an oxidative burst (possibly mediated or amplified by SA inhibition of catalase and APX) is followed by a more reducing environment that (potentially in conjunction with thioredoxin TRX-h5) causes the NPR1 oligomer to disassociate (Mou et al. 2003; Tada et al. 2008). NPR1 conformation also appears to be regulated by NO, although it is currently unresolved whether S-nitrosylation promotes the formation of NPR1 oligomers or monomers (Lindermayr et al. 2010; Tada et al. 2008). In addition, direct binding of SA by NPR1 promotes its monomerization (Wu et al. 2012). NPR1 monomers are then transported to the nucleus. In contrast to the inoculated leaf, the systemic

leaves of SAR-induced plants accumulate much lower concentrations of SA (Klessig et al. 2016). In these distal leaves but not in the locally infected leaves, NPR1 nuclear transport requires SNF1-RELATED PROTEIN KINASE 2.8 (SnRK2.8), a serine (Ser) and threonine (Thr) kinase that phosphorylates NPR1 at Ser589 and possibly Thr373 (Lee et al. 2015).

Once in the nucleus, NPR1 coactivates the transcription of defense-associated genes, such as *PR-1*, by directly interacting with basic leucine zipper (bZIP) TFs from the TGA family (Birkenbihl et al. 2017; Gao et al. 2015; Herrera-Vásquez et al. 2015; Pajeroska-Mukhtar et al. 2013; Shigenaga and Argueso 2016). Additionally, *PR-1* expression may be modulated by interactions between NPR1 and members of the NIM1-INTERACTING family and by proteins thought to function downstream of NPR1 (Fu and Dong 2013; Hermann et al. 2013; Pajeroska-Mukhtar et al. 2013; Seyferth and Tsuda 2014). WRKY TFs play important roles in positively or negatively regulating immune responses and several are direct transcriptional targets of NPR1 (Fu and Dong 2013; Seyferth and Tsuda 2014). WRKY binding sites, termed W boxes, are located in the promoters of many downstream defense genes, such as *PR-1*, as well as in those of *NPR1* and *ICS1*. This latter finding suggests the presence of a feedback loop that can fine-tune SA biosynthesis and signaling. Recently, phosphorylation of NPR1 at Ser11/Ser15 was shown to enhance its sumoylation by SMALL UBIQUITIN-LIKE MODIFIER 3, whereas phosphorylation at Ser55/Ser59 suppressed its sumoylation (Saleh et al. 2015). Strikingly, sumoylation reduced the ability of NPR1 to interact with WRKY70, a transcriptional repressor of *PR-1* and promoted its interaction with TGA3, thereby activating defense gene expression (Saleh et al. 2015). Sumoylation also enhanced the ability of NPR1 to interact with NPR3 and NPR4, which target NPR1 for proteasome-mediated degradation (Fu et al. 2012; Saleh et al. 2015; Spoel et al. 2009). By coupling NPR1 activation with NPR1 degradation, sumoylation provides a mechanism for ensuring that defense signaling is transient.

TARGETED APPROACHES TO IDENTIFY SA RECEPTORS

The discovery that several members of the NPR family bind SA has provided additional, critical insights into how NPR1-dependent SA signaling is mediated (Fu et al. 2012; Wu et al. 2012). The role of NPR1 as a key regulator of immunity raised the possibility that it was a SA receptor (Kumar 2014; Kuai et al. 2015; Pajeroska-Mukhtar et al. 2013; Yan and Dong 2014). However, initial efforts to identify a direct interaction between SA and NPR1 were unsuccessful. By coupling NPR1 to a solid phase and employing equilibrium dialysis, Wu et al. (2012) demonstrated that NPR1 binds [¹⁴C]SA with a K_d of 140 nM. The high affinity of NPR1 for SA (K_d of 191 nM) was subsequently confirmed using classical size exclusion chromatography with [³H]SA as well as by assessing its ability to be crosslinked to the photo-reactive SA analog 4-azidoSA and to bind to the SA derivative 3-aminoethylSA (Manohar et al. 2015). Yue Wu and coworkers reported that two aa residues located in the C-terminal transactivation domain (Cys521/Cys529) were required for SA binding, as was the transition metal copper. SA binding was proposed to regulate NPR1 activity as a transcription coactivator by i) triggering NPR1 oligomer disassembly and ii) altering the conformation of the NPR1 transactivation domain, thereby releasing it from the autoinhibitory BTB/POZ domain. It should be noted that Cys 521/Cys 529 are not universally conserved in NPR1 orthologs. Whether SA binds and regulates these proteins through a similar mechanism via

interactions with other aa residues capable of binding a metal cofactor is currently unclear.

In comparison with the above model, another study suggested that the SA receptor was not NPR1 but, rather, its paralogs NPR3 and NPR4 (Fu et al. 2012). NPR3 and NPR4 are adaptor proteins of the CULLIN 3 (CUL3) E3 UBIQUITIN LIGASE, and they specifically target NPR1 for proteasome-mediated degradation. In vitro analyses revealed that the interaction between NPR1 and NPR3 or NPR4 was enhanced or disrupted, respectively, by the presence of SA. Furthermore, both proteins bound SA and active but not inactive SA analogs, with NPR4 exhibiting a K_d of 46.2 nM for SA and NPR3 a K_d of 981 nM. Based on these findings, it was proposed that in uninfected cells, which contain low levels of SA, NPR4-CUL3-mediated degradation continuously clears NPR1 from the nucleus to prevent spurious activation of immune signaling. Following pathogen infection, rising SA levels disrupt the NPR4-NPR1 complex, thereby releasing active NPR1. In the infected and immediately surrounding cells, high SA levels promote NPR3-NPR1 complex formation, which reinitiates NPR1 turnover and may trigger HR formation. Since SA levels decrease in a gradient from the infection site, cells further removed accumulate intermediate levels of SA. NPR1 accumulation and immune signaling would presumably occur in these cells, because intermediate SA levels would be sufficient to release NPR1 from NPR4 but insufficient to promote NPR3-mediated degradation.

GENOME-WIDE, HIGH-THROUGHPUT SCREENS FOR SABPS

The development of genome-wide, high-throughput screens for identifying SABPs by Klessig and coworkers has led to significant advances in elucidating SA's mechanisms of action. These screens involve crosslinking the photo-reactive SA analog 4-azidoSA to proteins in soluble leaf extracts or on a protein microarray, followed by immuno-selection or -identification with high-affinity, anti-SA antibodies, respectively (Manohar et al. 2015; Moreau et al. 2013; Tian et al. 2012, 2015). Currently, these high-throughput strategies have identified 26 SABPs from *Arabidopsis* (The Arabidopsis Salicylic acid signaling network). In conjunction with other efforts, 40 SABPs have been identified in *Arabidopsis*, tobacco, tomato, and potato, some of which are paralogs, orthologs, or homologs.

Since it is generally assumed that plant hormones, like their animal counterparts, interact with one or a small number of receptors to elicit their effects, the identification of so many SABPs raises difficult questions as to whether all or just some should be designated SA receptors. To clarify the nomenclature, it was proposed that proteins whose function or activity is altered by binding of the corresponding hormone (or other ligand) be designated 'targets' (Klessig et al. 2016). A subset of these would then be designated receptors if they meet additional criteria. Unfortunately, deciding these criteria is problematic. SA binding alters the activity of many SABPs, making it a poor criterion for discriminating receptor status. Likewise, SA affinity is not an obvious criterion. Some SABPs, such as a few of the MeSA esterases (Du and Klessig 1997; Vlot et al. 2008), display a high affinity for SA similar to that of the putative receptors NPR1 and NPR4, whereas others exhibit a lower affinity similar to that of NPR3. Further complicating efforts to clarify the roles SABPs play is the observation that basal SA levels vary substantially between different plant species (Klessig et al. 2016). SA levels also vary within a given plant, depending on the subcellular compartment and tissue type, the developmental stage of the plant, and whether it is responding to abiotic or biotic stress. Thus, it is possible that

numerous SABPs are required to mediate myriad effects of SA, with their specific role determined by their location within the plant, their affinity for SA, and the local SA concentration. This scenario represents a paradigm shift for how, at least certain, hormones function. Future studies may reveal that this novel paradigm is applicable to other hormones in plants and, potentially, in animals. Indeed, while the primary mode of action of aspirin in humans has been ascribed to inhibition of the cyclooxygenases COX1 and COX2, several lines of evidence argue that additional SA/aspirin targets exist (Klessig et al. 2016).

The possibility that multiple SABPs are present in animals and that some of these targets are shared with plants was assessed by performing similar genome-wide, high-throughput screens to identify SABPs in human cells (Klessig et al. 2016). Several human SABPs were identified. Of these, GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE (GAPDH) (Choi et al. 2015a) and HIGH MOBILITY GROUP BOX 1 (HMGB1) (Choi et al. 2015b) have plant counterparts that also are SABPs (Choi et al. 2016; Tian et al. 2015). In *Arabidopsis*, which contains seven GAPDH isoforms distributed in different cellular compartments, five isoforms were identified as SABPs (Tian et al. 2015). GAPDH is a ubiquitous cytosolic enzyme that catalyzes a key step in glycolysis. GAPDH also appears to be involved in several pathological processes, including neurodegenerative diseases in humans and viral replication in both plants and humans (Klessig et al. 2016). These ‘moonlighting’ functions are suppressed by SA, as SA binding i) suppresses the ability of human GAPDH to translocate to the nucleus and induce cell death (Choi et al. 2015a) and ii) suppresses binding of human and plant GAPDHs to the genomes of certain viruses, which prevents efficient viral replication (Klessig et al. 2016; Tian et al. 2015). Human HMGB1 and its plant counterparts function in the nucleus as nonhistone, chromatin-binding proteins that regulate chromatin condensation and nucleoprotein complex formation. These proteins also are damage-associated molecular patterns (DAMPs) that trigger immune responses when they are released to the extracellular milieu by tissue damage or necrosis (Choi et al. 2015b, 2016). Strikingly, the DAMP activities of both human HMGB1 and *Arabidopsis* HMGB3 were suppressed by SA binding. Additional studies have revealed that members of the MICRORCHIDIA (MORC) protein family in plants and animals are SABPs (Manohar et al. 2017a). These proteins appear to play critical roles in gene silencing and disease progression (Koch et al. 2017). SA binding inhibits the topoisomerase II-like activities of plant and human MORCs (Manohar et al. 2017a). Together, these findings suggest that multiple disease-associated SA targets are shared in plants and animals.

CROSSTALK BETWEEN THE SA SIGNALING PATHWAY AND THOSE OF OTHER HORMONES DURING IMMUNE SIGNALING

Based on a substantial body of evidence, primarily generated from studies of Arabidopsis, the SA and JA signaling pathways are thought to comprise the backbone of plant immunity (Pieterse et al. 2009, 2012; Shigenaga and Argueso 2016). SA usually mediates resistance to biotrophic pathogens, whereas JA activates resistance to herbivorous insects and necrotrophic pathogens. ET often works synergistically with JA to mediate resistance to necrotrophs. In general, the SA and JA/ET pathways are mutually antagonistic; however, synergistic interactions between these pathways have been noted (Caarls et al. 2015; De Vleeschauwer et al. 2014; Pieterse et al. 2012; Shigenaga and Argueso 2016; Shigenaga et al. 2017). Indeed, JA synthesis and signaling was recently shown to positively regulate RPS2-mediated ETI to a hemibiotrophic bacterial pathogen in Arabidopsis (Liu et al. 2016). Hormones

traditionally associated with plant growth and abiotic stress responses, including auxins, abscisic acid (ABA), cytokinins, gibberellins, and brassinosteroids, also have been shown to play important roles in plant immune signaling. These hormones generally influence immunity by tilting the hormonal balance toward SA or JA, thereby modulating the SA-JA signaling backbone. Further adding to the complexity of immune signaling, certain pathogens downregulate the correspondingly more effective SA or JA signaling pathway by hijacking the antagonistic hormone pathway either via effectors that target hormone signaling components or by producing plant hormones or hormone mimics. Through this process, they enhance host susceptibility. As these topics have been reviewed extensively (De Vleeschauwer et al. 2014; Denancé et al. 2013; Pieterse et al. 2012; Robert-Seilanianantz et al. 2011; Shigenaga and Argueso 2016; Verma et al. 2016; Yang et al. 2015), they will not be covered further here.

Recent efforts to unravel the relationship between immune signals have focused on analyses of combinatorial *Arabidopsis* mutants defective for JA, ET, SA, and PAD4, singly or in combination (Hillmer et al. 2017; Kim et al. 2014; Mine et al. 2017; Tsuda et al. 2009, 2013). ***These studies revealed that immune signaling is not mediated by individual pathways but, rather, by a complex network in which the JA, ET, SA, and PAD4 sectors crosstalk extensively.*** The crosstalk in this network is hypothesized to provide robust immune signaling even when various signaling sectors are disrupted by pathogen effectors or mutations. It also confers a tunable response that not only is appropriate for the type of attacking pathogen but also prevents activation by MAMPs associated with nonpathogenic microbes.

Although the mechanisms through which immune signaling pathways crosstalk are not well understood, several SABPs have been implicated in this process. For example, cytoplasmic NPR1 is required for SA-mediated suppression of the JA signaling pathway (Spoel et al. 2003). By contrast, NPR3 and NPR4 but not NPR1 mediate SA-dependent activation of JA signaling and synthesis during RPS2-triggered ETI (Liu et al. 2016). SA enhances the interaction between NPR3/NPR4 and several JASMONATE ZIM DOMAIN proteins. By targeting these repressors of JA signaling for proteasome-mediated degradation, SA activates the JA pathway. SABPs also have been implicated in mutually antagonistic crosstalk between the SA and ABA signaling pathways. ABA suppresses SA signaling by enhancing NPR3- and NPR4-mediated degradation of NPR1 (Ding et al. 2016b). Conversely, SA appears to suppress ABA signaling by binding members of the TYPE 2C PROTEIN PHOSPHATASE (PP2C) protein subfamily (Manohar et al. 2017b). PP2Cs repress the ABA pathway by dephosphorylating (and thereby inactivating) members of the SnRK2 family, which are positive regulators of ABA signaling. In the presence of ABA, PP2Cs tightly bind ABA receptor proteins. This inhibits PP2C phosphatase activity and, thus, allows SnRK2s to relay the ABA signal. The discovery that PP2Cs are SABPs and that SA i) suppresses the ABA-enhanced interaction between PP2Cs and ABA receptor proteins and ii) enhances PP2C stability in vitro suggests that the SA-PP2C interaction antagonizes ABA signaling through more than one mechanism. Another recent study indicated that the *Arabidopsis* CAT2 protein mediates antagonistic crosstalk between the SA, JA, and auxin (IAA) signaling pathways (Yuan et al. 2017). SA-mediated inhibition of CAT2 led to increased H₂O₂ levels, led to sulfenylation, thus suppression of TRYPTOPHAN SYNTHETASE β SUBUNIT 1. Since this enzyme generates the auxin precursor tryptophan, the IAA level was reduced. SA binding by CAT2 also reduced JA accumulation by preventing CAT2 from directly interacting with (and stimulating) the activities of two peroxisomal

CONCLUSIONS AND FUTURE DIRECTIONS

Since Kenneth Chester first published his observations on acquired resistance in 1933, our understanding of how plants activate immune responses after pathogen infection has increased tremendously. Much of this progress has occurred over the past three decades, sparked by the discovery in 1990 that SA is an endogenous signal for disease resistance. Efforts to elucidate SA-mediated immune signaling have identified two pathways through which SA can be synthesized as well as multiple proteins involved in regulating SA synthesis and metabolism. In addition, biochemical, molecular, and genetic approaches have identified some of the downstream signaling components and mechanisms through which the SA signal is transduced. Superimposed on these findings is the growing recognition that SA is just one player, albeit a critical one, in a complex signaling network that orchestrates immune responses. While SA, JA, and ET form the backbone of defense signaling, this process is influenced by many other plant hormones as well as by certain pathogen effectors. Adding to the complexity of immune signaling is the discovery that the SA- and JA/ET-mediated defense pathways can interact in an antagonistic, synergistic, or compensatory manner. This complexity presumably helps tailor defense responses to maximize their effectiveness against pathogens with different lifestyles, while also ensuring a robust immune response despite potential disruptions in specific immune signaling pathways.

Despite this progress, there are significant gaps in our knowledge. Future studies are required to clarify how pathogen perception by PRRs and R proteins is transduced into the activation of early cellular responses and SA synthesis. In addition, while multiple mobile SAR signals have been identified, their role in activating SA-dependent immune responses in the systemic leaves is only partially understood. The enzymes responsible for SA biosynthesis via the PAL and IC pathways also have not been fully elucidated, and the roles these pathways play in different plant species is unclear. The regulation of *ICS1* expression in the nucleus and the mechanisms through which retrograde signals from the chloroplast influence this process also need to be clarified. Investigating the signaling mechanisms that operate downstream of SA is another important topic for future studies. In addition to identifying novel SA targets and receptors, determining how the current trove of SA targets and receptors impacts this process should provide important clues into how SA exerts its myriad effects.

Beyond the SA pathway, developing a comprehensive understanding of immune signaling will require elucidating the complex crosstalk between SA and other plant hormones. Given that defense responses are activated by an intricate signaling network, the traditional strategy of assessing gene and pathway function by analyzing mutants lacking a single gene or pathway may be of limited utility, as the loss of one might be buffered by another located elsewhere in the network. Instead, future studies will require generating complex combinations of mutants, such as those used to investigate the interactions between the SA, JA, ET, and PAD4 pathways. The results of these studies can then be used to develop increasingly sophisticated models to predict signaling dynamics. In turn, these models can be used to identify important signaling hubs and assess how they fine-tune SA-dependent immune responses. Based on their combinatorial mutant analyses, Fumiaki Katagiri and coworkers have already built a model that predicts the signaling dynamics between the SA, JA, ET, and PAD4 pathways during PTI (Kim et al. 2014). While these efforts represent tremendous

progress, the molecular mechanisms through which the SA, JA, ET, and PAD4 pathways modulate each other and how these interactions activate defenses that are optimized to combat different types of pathogens (and herbivores) is largely unknown. It also should be noted that the SA, JA, ET, and PAD4 sectors govern up to 80% of PTI or ETI, depending on the MAMP or pathogen, respectively (Tsuda et al. 2009). Thus, additional signaling sectors remain to be identified and their mechanisms of action and relationship to the SA, JA, ET, and PAD4 sectors investigated. Superimposed on these studies, it will be necessary to elucidate how other plant hormones influence immune signaling. In conjunction with combinatorial mutant analyses, investigating the SABPs implicated in hormone crosstalk should be highly informative.

In addition to benefiting basic research, the results of these studies have practical applications for agriculture. Currently, the use of synthetic pesticides and fungicides enables growers to boost crop yield, maintain high crop quality, and ensure production stability. However, many of these compounds are toxic and pathogen resistance can develop if they are overused. The ability to manipulate SAR effectively in crop species would provide farmers and growers with an environmentally friendlier strategy for preventing crop loss by reducing reliance on synthetic pesticides and fungicides. SA treatment effectively induces SAR in many plant species; however, it is too phytotoxic for widespread use (Conrath et al. 2015). Several synthetic compounds that induce SAR to a similar range of pathogens as SA have been identified, including INA, BTH, PBZ, and the PBZ active metabolite 1,2-benzisothiazol-3(2H)-one 1,1-dioxide (Conrath et al. 2015; Gozzo and Faoro 2013; Walters et al. 2013). When applied at high concentrations, these SA synthetic or functional analogs directly induce defense responses. By contrast, low concentrations of these compounds prime immunity, as they induce little to no plant response until pathogen infection, at which time defense responses are triggered either more rapidly, strongly, or both (Conrath et al. 2006). In the field, these SA synthetic or functional analogs as well as several compounds that promote resistance, at least in part, by priming or inducing SAR can induce broad-spectrum, long-lasting resistance (Dempsey and Klessig 2017). However, the level of resistance is variable, with disease reduction ranging from 20 to 85% (Walters et al. 2013). Other issues growers must consider include i) a possible reduction in plant fitness due to the activation of defense responses, although this issue can be minimized by priming rather than directly inducing defenses, ii) variable levels of efficacy, depending on the plant cultivar and dosage, and iii) the possibility that activating SA-induced defenses will suppress JA signaling and, thereby, enhance crop susceptibility to necrotrophic pathogens (Gozzo and Faoro 2013; Walters et al. 2013).

As our understanding of the SA signaling pathway and its interactions with other immune signaling sectors increases, these findings may provide clues as to how SAR-inducing compounds can be modified to improve their performance without sacrificing plant fitness. In addition, they may identify novel signaling components (within the SA pathway or an interacting sector) that could serve as useful targets for the next generation of SAR-inducing agrochemicals. The development of models that can predict how the immune signaling sectors interact should also clarify how plants respond effectively to pathogens with different lifestyles and, critically, to mixed infections by more than one pathogen. These models should also provide insights into how plants balance the competing demands of growth, immunity, and abiotic stress. In the field, plants are often confronted with multiple biotic and abiotic challenges simultaneously. Thus, this knowledge should help growers devise an effective strategy that maximizes the potential of SAR-inducing compounds to control

disease and maintain high crop yields, while reducing dependence on synthetic pesticides and fungicides. Finally, the discovery that plants and animals have several disease-associated SA targets in common suggests that elucidation of the plant immune signaling network may yield discoveries that are applicable to both kingdoms. Beyond providing insights into how SA elicits its effects, these results may suggest novel strategies for controlling pathological processes in both kingdoms.

NOTE ADDED IN PROOF

Two just published papers report that FMO1 hydroxylates Pip to form N-hydroxypipecolic acid, which is a mobile signal for SAR induction (Chen et al. 2018; Hartmann et al. 2018).

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AUTHOR-RECOMMENDED INTERNET RESOURCE

The *Arabidopsis* Salicylic acid signaling network:
<http://bioinfo.bti.cornell.edu/SA2010>