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Systemic Acquired Resistance

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ABSTRACT

Systemic acquired resistance (SAR) is a mechanism of induced defense that confers long-lasting protection against a broad spectrum of microorganisms. SAR requires the signal molecule salicylic acid (SA) and is associated with accumulation of pathogenesis-related proteins, which are thought to contribute to resistance. Much progress has been made recently in elucidating the mechanism of SAR. SAR confers quantitative protection against a broad spectrum of microorganisms in a manner comparable to immunization in mammals, although the underlying mechanisms differ. Discussed here are the molecular events underlying SAR: the mechanisms involved in SAR, including lignification and other structural barriers, pathogenesis-related proteins and their expression, and the signals for SAR including salicylic acid. Recent findings on the biological role of system in, ethylene, and electrical signals are reviewed.

Key Words: Plant defense, salicylic acid, SAR, NPR1, TGA factor.

Introduction

Plants have evolved a number of inducible defense mechanisms against pathogen attack. Recognition of a pathogen often triggers a localized resistance reaction, known as the hypersensitive response (HR), which is characterized by rapid cell death at the site of infection (Hammond et al. 1996). In the 1960s, Ross showed that tobacco plants challenged with tobacco mosaic virus (TMV) subsequently developed increased resistance to secondary infection in distal tissues (Ross 1961). Molecularly, SAR is characterized by the increased expression of a large number of pathogenesis-related genes (*PR* genes), in both local and systemic tissues. PR proteins were first described in the 1970s by Van Loon, who observed accumulation of various novel proteins after infection of tobacco with TMV (Van Loon et al. 1970; Van Loon et al. 1999). In 1979, White observed that PR protein accumulation and resistance to TMV could be induced by treatment of tobacco with salicylic acid (SA), aspirin (acetyl SA), or benzoic acid (White 1979). Evidence that SA is a signal for the induction of SAR came from two studies published in 1990 (Malamy *et al*, 1990; M´etraux et al. 1990). Malamy *et al*. showed that the endogenous SA concentration rises in both local and systemic tissues after infection of tobacco with TMV and this rise correlates with *PR* gene induction (Malamy et al. 1990) found that cucumber plants infected with either *Colletotrichum lagenarium* or tobacco necrosis virus (TNV) have considerably elevated levels of SA in the phloem sap (M´etraux *et al*, 1990). In a search for SA analogues that were less phytotoxic than SA, 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole S-methyl ester (BTH) were found to induce the same set of *PR* genes (Friedrich *et al*,

1996; G¨orlach et al. 1996; Lawton et al. 1996; M´etraux et al. 1991; Ward et al. 1991). In the past 10 years, genetic analyses in the model plant *Arabidopsis* have identified additional components of SAR downstream of SA. Plants that are nonresponsive to SA were identified in a number of mutant screens and found to have mutations in the same gene, *NPR1*/*NIM1* (*NON-EXPRESSER OF PR GENES1/ NONINDUCIBLE IMMUNITY1*) (Cao et al. 1994; Delaney et al. 1995; Glazebrook et al. 1996; Shah et al. 1997). The observations that even susceptible plants can mount some degree of defense against pathogens plays into the overall concept that plants come equipped with defense genes. This form of defense, known as basal disease resistance (Jones and Dangl 2006), is induced in susceptible plants upon infection with compatible pathogens. Although not effective enough to stop the pathogen, basal defenses may help limit the spread of the disease in the infected tissue. These defenses are likely the same as those induced in other forms of resistance, though they may often be expressed too late or at too low a level to be totally effective (phytoalexin accumulation is one good example of this type of defense response) (Hammerschmidt 1999b). It is important to note that the induced plants may still become diseased, indicating that induced resistance does not provide the level of resistance mediated by major R genes. Depending on the type of inducing agent and the signaling pathways involved, induced resistance can be classified in different ways. The two forms of induced resistance that have been best characterized are systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Van Loon et al. 1998). However, it is likely that other forms of induced resistance exist. The essential elements of the phenomenology of SAR have already been described (Hammerschmidt and kuc 1995; Kessmann et al. 1994; Madamanchi and kuc 1991; Schneider et al. 1996). This type of resistance is expressed against a broad spectrum of organisms, which may differ from the SAR-inducing organism. In cucumber, for example, a primary inoculation with the fungus *Colletotrichum lagenarium*, the causal agent of anthracnose, induces SAR against a dozen diseases caused by fungal and bacterial as well as viral pathogens (Hammerschmidt and kuc 1995; Kessmann et al. 1994; Madamanchi and kuc 1991; Schneider et al. 1996). In most cases, the first inoculation leads to localized necrosis (Madamanchi and kuc 1991). In gene-for-gene resistance, a plant is either resistant or susceptible against certain races of a pathogen, whereas SAR confers quantitative protection against a broad spectrum of microorganisms. The time needed for the establishment of SAR depends on both the plant and the type of inducing organism. A very rapid induction was reported for cucumber, where SAR sets in as early as 7 h after a primary inoculation with *Pseudomonas syringae* (Smith and M´etraux 1991). Injection of spores of the blue mold pathogen, *Peronospora parasitica* pv *tabaci*, under the epidermis of the stem of tobacco plants leads to the expression of SAR in the leaves against the same fungus 2–3 weeks after the primary inoculation (Cohen and kuc 1981). The level of protection may vary depending on the organism used for the primary inoculation and particularly on the extent of the necrosis (Madamanchi and kuc 1991). An interesting case of acquired resistance was described using plant growth promoting rhizobacteria (PGPR) colonizing the rhizosphere as biocontrol agents. PGPR applied to the soil remain localized at the root surface and can induce resistance in the leaves or the stem. Evidence from several experimental systems indicates that PGPR can protect plants systemically against various pathogens without causing any symptoms (Buhot et al. 2001; Liu et al. 1995; Maurhofer et al. 1994; Van et al. 1991). To differentiate this form of acquired resistance from SAR, it has been termed induced systemic resistance (ISR) (Pieterse et al. 1996). A number of articles have focused on the reactions taking place after infection at the site of primary pathogen attack (Dangl et al. 1996; Dixon et al. 1994; Hammond-Kossack and Jones 1996; Jones and Dangl 1996), as well as on SAR (Hunt and Ryals 1996, Kessmann et al. 1994; Ryals et al. 1996; Schneider et al. 1996). In this review, we discuss the reactions leading to SAR and the endogenous signals involved in their activation.

THE SIGNALS FOR SYSTEMIC ACQUIRED RESISTANCE

Salicylic Acid

DISCOVERY In 1979, White observed that treatments with SA can decrease the disease symptoms caused by TMV in the tobacco cultivar Xanthi-nc and can lead to accumulation of PRs (White 1979).The detection of increased SA levels in systemic leaves and in the phloem led many researchers to believe that SA might be a systemic signal for SAR. The evidence for and against this hypothesis has been the subject of previous reviews (Dempsey et al. 1999; Shah and Klessig 1999). Labeling studies in TMV-infected tobacco showed that most of the SA (69%) accumulating systemically was made and exported from the inoculated leaf (Shulaev et al. 1995). Similarly, in cucumber infected with TNV, SA found in systemic leaves was both imported from the infected leaf and synthesized de novo (Meuwly et al. 1995; M¨olders et al. 1996). A more recent study suggests that signaling might occur through the conversion of SA to the volatile compound methyl salicylate, which could induce resistance

not only in the uninfected parts of the same plant but also in neighboring plants (Shulaev et al. 1997). Furthermore, grafting experiments in tobacco between wild-type scions and *nahG*-expressing rootstocks showed that, although the rootstock was unable to accumulate SA, the SAR signal was still produced and translocated to the scion (Vernooij et al. 1994). SAR can be broadly defined as a form of induced resistance that is activated throughout a plant typically following infection by a pathogen that causes localized necrotic lesions. The necrosis can be the result of disease induced by a pathogen or a hypersensitive response (HR) (Kuc´ 1982; Kuc´ et al. 1975; Ross 1961b). Multiple rounds of inducing inoculations (‘‘booster’’ inoculations) can also increase the level of SAR (Kuc´ 1982). SAR is dependent on salicylic acid (SA) signaling (Gaffney et al. 1993). Although the role of SA as a mobile signal for SAR is still debatable (Rasmussen et al. 1991; Shulaev et al. 1995; Vernooij et al. 1994), there is little doubt that this simple phenol is essential for the expression of SAR (Delaney et al. 1994). Similarly, the application of SA to various plants also induces SAR genes (Bol et al. 1990; Bowles 1990; Cutt and Klessig 1992; Kessmann et al. 1994; Linthorst 1991; Madamanchi and Kuc 1991; Schneide et al. 1996; van Loon et al. 1994). Van Loon first raised the possibility in 1983 of a link between SA and SAR; he suggested that ethylene-induced accumulation of PRs is mediated in the plant by the synthesis of “an aromatic compound that mimics the action of SA” (van Loon 1983). It was only in 1990 that two laboratories working independently postulated that SA could be a putative endogenous signal for SAR (Malamy et al. 1990; M´etraux et al. 1990). This hypothesis was based on the observation that the endogenous level of SA increases locally and systemically in tobacco plants inoculated locally with TMV (Malamy et al. 1990). SA also increases in the phloem of infected cucumber before the expression of SAR, consistent with a role as a signal for SAR (M´etraux et al. 1990; Rasmussen et al. 1991). In plants transformed with the *NahG* gene (naphthalene hydroxylase G), the SA levels are low and SAR is blocked, which indicates that SA is required for SAR induction (Delaney et al. 1994; Gaffney et al. 1993). These studies show that depletion of SA affects gene-forgene resistance (Delaney *et al*, 1994; Gaffney et al. 1993). The importance of SA in gene-for-gene resistance is further demonstrated using 2-amino-indane-2-phosphonic acid (AIP), an inhibitor of PALactivity and of the phenylpropanoid biosynthetic pathway leading to SA. The normally incompatible interaction between the Arabidopsis Col-0 ecotype and *Peronospora parasitica* isolate EMWA becomes compatible after treatment of Arabidopsis with AIP. Exogenously supplied SA counteracts the effect of AIP. Thus both PAL activity and SA are required for the resistance gene-mediated defense response (Mauch-Mani and Slusarenko 1996). Further evidence for the role of SA in SAR comes from work with Arabidopsis mutants. The *cim3* mutant exhibits *c*onstitutive *im*munity against virulent pathogens without any detectable lesions, accumulates constitutive levels of mRNAs for the SAR markers PR-1, PR-2, and PR-5, as well as elevated levels of free and conjugated SA (Ryals et al. 1996). The importance of SA is demonstrated by expressing the *nahG* gene in *cim3*: In this case both constitutive immunity and constitutive expression of the SAR genes are lost (Ryals et al. 1996). A number of conjugated forms of salicylates have been identified in various plants (Lee et al. 1995; Pierpoint 1994). SA formed endogenously in tobacco after TMVinfection or SA accumulating after feeding is converted into SA-glucoside, mainly in the form of a 2-¯-D-glucosyl conjugate (Enyedi et al. 1992; Hennig et al.1993). SA 2-β-D-glucoside was, however, not detected in phloem exudates of TMV-inoculated tobacco plants (Enyedi et al. 1992), which suggests that this is not the main translocated form of SA. The conversion of SA to a 2-β-D-glucosyl conjugate is also observed in rice (Silvermann et al. 1995). Feeding tobacco discs with [7-14C]-SA shows that besides 2-D-glucoside SA, minor amounts of SA glucose ester are also produced (Edwards et al. 1994). Considerable attention has been given to the action of SA with respect to the initial necrotization event. Is SA a cause or a consequence of cell death? SA can be phytotoxic, but when applied exogenously at optimal levels it can induce SAR without lesion formation. Also, in *nahG*-expressing plants where the endogenous level of SA is low, lesion formation is not impaired (Delaney et al. 1994; Gaffney et al. 1993). To dissect the pathway from the initial necrosis to the expression of SAR, mutants of Arabidopsis constitutively expressing lesions have been studied. Several *lsd* and one *acd* mutant display necrotic lesions, accumulate high amounts of SA, and express high levels of mRNA for SAR genes as well as increased resistance toward pathogens (Dietrich et al. 1994; Greenberg et al. 1994; Weymann et al. 1995). A final characteristic of SAR is that the resistance is effective against a broad range of pathogens that include bacteria, true fungi, oomycetes, and viruses (Deverall 1995; Hammerschmidt and Kuc´ 1995; Kuc´ 1982). Within this range, recent studies with model systems (Arabidopsis thaliana) suggest that SAR, and SA-mediated resistance in general, may be most effective against biotrophic and hemibiotrophic pathogens and not against necrotrophs (Glazebrook 2005; Oliver and Ipcho 2004). However, as discussed later in this review, SA induces resistance to viruses by an NPR1-independent mechanism (Singh et al. 2004).

Lipid-Based Signal Molecule

Exciting new work suggests that a lipid-based molecule may be the mobile signal for SAR. Maldonado et al. showed that the *dir1* (*defective in induced resistance 1*) mutant has normal local resistance to pathogens but is unable to develop SAR or express *PR* genes in systemic leaves (Maldonado et al. 2002). The similarity of DIR1 to LTPs suggests that the mobile signal for SAR might be a lipid molecule. LTPs form a multigene family in *Arabidopsis* with 71 predicted members (Beisson et al. 2003). Interestingly, they share sequence similarity with elicitins from *Phytophthora* spp, which are elicitors of plant defense responses (Blein et al. 2002). The extracellular location of LTPs and elicitins is consistent with a role in signaling and implies the presence of plasma membrane (PM) receptors involved in signal transduction. Indeed, wheat LTP1 binds to the same PM receptor as the *Phytophthora* elicitin cryptogein (Buhot et al. 2001). Further evidence for a lipid-based signal molecule comes from the characterization of the *eds1* and *pad4* mutants, which are both defective in lipase-like proteins (Falk et al. 1999; Jirage et al. 1999). It was subsequently discovered that *pad4* weakens local resistance mediated by the same subset of *R* genes that are blocked by *eds1* (Feys et al. 2001). These *R* genes encode TIR-NB-LRR-type resistance proteins. However, many other *R* genes act through an EDS1-independent signaling pathway (Aarts et al. 1998). In *eds1* and *pad4* plants, even when a normal HR is elicited by pathogens that trigger the EDS1-independent pathway, SAR cannot be induced (L. Jorda & J. Parker, personal communication). Experiments using phloem exudates have shown that EDS1 is required for both production of the mobile signal in the local tissue and perception of the signal in the systemic tissue (C. Lamb, personal communication). Recently, it was discovered that a tobacco SA-binding protein, SABP2 (Du and Klessig 1997), is also a lipase and that its lipase activity is increased four- to fivefold by addition of SA (Kumar and Klessig 2003).

Reactive Oxygen Species

Early studies could detect no reactive oxygen species (ROS) production in systemic tissues during the onset of SAR (Neuenschwander et al. 1995; Ryals et al. 1995). However, it has since been discovered by Alvarez et al. that H2O2 accumulates in small groups of cells in uninoculated leaves of *Arabidopsis* after infection with an avirulent strain of *P. syringae* (Alvarez et al. 1998). These microbursts occur within two hours after an initial oxidative burst in the inoculated tissue and are followed by the formation of microscopicHRlesions. Using catalase to scavenge H2O2, or DPI (diphenylene iodonium) to inhibit the NADPH oxidase, it was demonstrated that both the primary and secondary oxidative bursts are required for the onset of SAR.

Systemin

After insect attack, plants respond with the accumulation of proteinase inhibitors in the wounded leaves and in distal unwounded leaves (Ryan 1990; Schaller and Ryan 1996). Proteinase inhibitors inhibit the activity of digestive proteases localized in the insect gut and can lead to malnutrition, reduced growth, and sometimes death of the feeding insects (Ryan 1990). The systemic induction of proteinase inhibitors has been most extensively studied in potato and tomato (Pena-Cortes et al. 1988; Ryan 1990), but has also been reported in other plant species including alfalfa (Brown et al.1985), melon (Roby et al. 1987), and maize (Cordero et al. 1994). The systemic signal has been isolated from tomato and consists of an 18-amino acid peptide called systemin. Systemin in amounts as low as femtomoles induces de novo synthesis of proteinase inhibitors when supplied to young tomato plants (Pearce et al. 1991). A synthetic peptide has full inducing activity (Pearce et al. 1991). Abscisic acid (ABA), JA, and systemin induce proteinase II inhibitor (Pin2) protein and gene expression in the treated leaves and in systemic leaves (Farmer and Ryan 1992; Pena-Cortes et al. 1995; Pena-Cortes et al. 1989). Upon wounding, there is an increase of ABA and JA levels (Pena-Cortes et al. 1993; Pena-Cortes et al. 1991). Radiolabeled [14C]-systemin applied to wounded tomato plants is distributed throughout the wounded leaf within 30 minutes and to the petiole, stem, and upper leaves within hours (Narvaez-Vasquez et al. 1995). The movement of [14C]-systemin is similar to the movement of [14C]-sucrose applied to leaf wounds, and the translocation of [3H]-systemin is inhibited by the sulfhydryl reagent p-chloromercuribenzenesulfonic acid, which is an inhibitor of the apoplasmic phloem loading and unloading of sucrose (Narvaez-Vasquez et al. 1994). Thus, translocation of systemin probably occurs by a mechanism similar to sucrose translocation from the apoplast into the phloem where it is systemically transported. Systemin is synthesized as a precursor protein of 200 amino acids, prosystemin, with the systemin sequence located near its C terminus (McGurl et al. 1992). Prosystemin does not possess a signal sequence for targeting to the secretory pathway and is probably stored in the cytoplasm (Schaller and Ryan 1996). Prosystemin mRNA is present throughout tomato plants except in roots and accumulates in leaves upon wounding (McGurl et al. 1992). Overexpression of the prosystemin gene in transgenic tomato plants results in constitutive expression of proteinase inhibitors in the absence ofwounding (McGurl et al. 1994). Grafting the

upper half of an untransformed tomato plant onto the lower half of a plant transformed with the prosystemin gene leads to constitutive expression of the proteinase inhibitor proteins in the whole plant. Thus, a mobile signal, systemin, is generated by the expression of the prosystemin transgene and travels from the lower transgenic part through the graft into the untransformed upper part, where it activates the proteinase inhibitor genes (McGurl et al. 1994). Tomato plants transformed with an antisense prosystemin cDNA show a great reduction of the expression of proteinase inhibitors after wounding (McGurl et al. 1992; Orozco-Cardenas et al. 1993). Leaves of tomato plants overexpressing a prosystemin gene also show enhanced levels of polyphenol oxidase, and supplying young tomato plants with systemin through cut stems induces polyphenol oxidase activity in leaves (Constabel et al. 1995). This enzyme is induced after wounding as well (Constabel et al. 1995). Systemin also induces synthesis of mRNA for an aspartic protease in tomato plants (Schaller and Ryan 1996). Recently, a systemin binding protein has been isolated from tomato leaf plasma membranes (Schaller and Ryan 1996). After spraying the lower part of the foliage of ABA-deficient potato plants with ABA, ABA levels increase in the distal nonsprayed tissues and *Pin2*mRNA accumulates (pena-Cortes et al. 1995). The ABA-deficient plants are unable to synthesize ABA de novo, and thus it is possible that exogenously applied ABA migrates to the nonsprayed tissue. However, Pearce & Ryan (Schaller and Ryan 1996) found that when ABA was supplied to young tomato plants, very little proteinase inhibitor protein accumulated compared to the levels reached after treatment with systemin. Thus, it seems that ABA is required for the wound response but would not behave as the primary wound signal. The hydraulic signals propagate changes in water pressure, which can be detected systemically using sensitive pressure transducers (Malone 1992). At wound sites, leaf cells are broken and the sap is released in the apoplasm. The sap and its solutes are drawn into a nearby intact xylem vessel (Malone et al. 1994).

Electrical Signals

Mechanical wounding of the cotyledons of young tomato plants leads to the slow (1–4 mm s¡1) transmission of an action potential out of the cotyledons and into the first leaf (Wildon et al. 1992). Application of electrical currents to tomato leaves leads to the accumulation of *Pin2* mRNA both locally and systemically, similar to induction by wounding or heat treatment (Herde et al. 1995; Pena-Cortes et al. 1995). However, the tension needed to induce *Pin2* accumulation (10 V) is much larger than the tension measured in the tissue after wounding (20 mV) (Wildon et al. 1992). Electrical stimulation as well as wounding lead to stomatal closure after 2–3 min, followed by a more pronounced closure after 10 min (Herde et al.1995; Pena-Cortes et al. 1995). The first fast response would correspond to the electrical signal reported (Wildon et al. 1992). In addition, there would be a second electrical/hydraulic component. After wounding of one of the cotyledons of tomato plants by heat, an electrical signal is produced that propagates at a rate of 2 mm s¡1 through the plant and is correlated with the induction of proteinase inhibitor activity in leaf 1 (Rhodes et al. 1996).

Ethylene

Ethylene is a volatile plant hormone derived from methionine and involved in numerous physiological processes (kende 1993). Ethylene is produced upon wounding or infection by pathogens as well as by treatment with elicitors of defense responses (Boller 1990; Grosskopf et al. 1991). Exogenous application of ethylene to tobacco carrying the N gene for resistance to TMV results in resistance to TMV marked by a decrease in the size of the necroses (van Loon and Antoniw 1982). Ethylene can induce some of the PRs such as ¯-1, 3-glucanase and chitinase (Abeles et al. 1971). Structural reinforcement of the cell wall such as lignification and accumulation of hydroxyproline-rich cell wall proteins are also enhanced by ethylene (Boller 1990). Although such results might suggest that ethylene is the signal involved in the induction of SAR (Boller 1990), several experimental results indicate that ethylene might not be directly linked to the induction of SAR. SAR-gene expression in ethylene-insensitive mutants of Arabidopsis is similar to that in wild-type plants (Bleeker et al. 1988; Chang et al. 1993), although ethylene enhances the effect of SA (Lawton et al. 1995; Lawton et al. 1994) and mediates pathogen-induced damages (Bent et al. 1992). Thus, it is unlikely that ethylene is the systemic signal for SAR, but it seems to modulate to some extent the expression of resistance. In TMV inoculated tobacco leaves ethylene seems to act as an intermediate in SA induced synthesis of chitinase (Raz and Fluhr 1993).

Transport of the Systemic Signal

How does the SAR signal travel throughout the plant? Girdling experiments suggested that the SAR signal produced in inoculated leaves travels in the phloem to upper leaves (Guedes et al. 1980; Ross 1966). If the mobile signal does travel through the phloem, the pattern of SAR induction should match the transport of sugars out of the infected leaf. When this was tested in *Arabidopsis*, it was observed that the movement of radioactively labeled sucrose did not exactly match the induction of SAR, SA accumulation, or *PR-1* expression (Kiefer et al. 2003). As described above, in many

plants SAR is preceded by an increase in SA concentration. However, some plants such as potato and rice have high endogenous levels of SA under non inducing conditions (Coquoz et al.1995; Silverman et al. 1995; Yu et al. 1997). Indeed, application of SA to potato does not protect it against *Phytophthora infestans* (Coquoz et al. 1995). However, expression of *nahG* in potato blocks resistance to *P. infestans* induced by arachidonic acid. This suggests that after treatment with arachidonic acid, instead of SA levels rising, the potato plants become more sensitive to SA (Yu et al. 1997).

THE ROLE OF SA IN SAR

The role of SA in SAR has been discussed extensively in a number of reviews (Dempsey et al. 1999; Dong 2001; Ryals et al. 1996; Shah and Klessig 1999.). As described above, in many plants SAR is preceded by an increase in SA concentration. However, some plants such as potato and rice have high endogenous levels of SA under noninducing conditions (Coquoz et al. 1995; Silverman et al. 1995; Yu et al. 1997). Indeed, application of SA to potato does not protect it against *Phytophthora infestans* (Coquoz et al. 1995).

SA Synthesis

It was previously assumed that SA for SAR is synthesized via the shikimatephenylpropanoid pathway (Lee et al. 1995), although this was never proven. It has recently been shown that, like bacteria, plants can also synthesize SA from chorismate via isochorismate. Expression of the bacterial enzymes catalyzing these reactions, isochorismate synthase 1 (ICS1) and isochorismate pyruvate lyase 1 (IPL1), in tobacco and *Arabidopsis* results in increased SA accumulation and pathogen resistance (Mauch et al. 2001; Verberne et al. 2000). Using HPLC, Nawrath & M´etraux isolated the SA induction-deficient *Arabidopsis* mutants *sid1* and *sid2*, which failed to accumulate SA after SAR induction (Nawrath et al. 1999). A recent breakthrough in our understanding of SA biosynthesis came when *SID2/EDS16* was cloned by Wildermuth et al. and shown to encode a putative chloroplast-localized ICS1 (Wildermuth et al. 2001). Since SA synthesis is not completely abolished in *sid2* plants, some SA must be produced either through the activity of another ICS-like protein, such as ICS2 (Wildermuth et al. 2001), or through the phenylpropanoid pathway. *Arabidopsis* ICS1 contains a putative plastid transit sequence, suggesting that SA synthesis occurs in the plastid. Interestingly, *EDS5/SID1* encodes another protein required for SA accumulation that has sequence similarity to the multidrug and toxin extrusion (MATE) family of transporter proteins (Nawrath et al. 2002).

Control of SA Synthesis

In plants such as tobacco and *Arabidopsis*, regulation of SA biosynthesis is an essential regulatory step in SAR activation. Therefore, identification of upstream regulatory components required for the induction of SA biosynthesis genes, especially *CS1*, will be an important step toward understanding the control of SAR. The induction of *ICS1* after infection by *Erysiphe orontii* and *P. syringae* pv. *Maculicola* is not affected by depletion of SA in *nahG* plants, indicating that the *ICS1* gene is not regulated by SA (Wildermuth et al. 2001). SA synthesis induced by another *R* gene, *RPS4*, requires *EDS1* and *PAD4* (Feys et al. 2001; Zhou et al. 1998). The *eds1* and *pad4* mutants also block SA synthesis triggered by infection with virulent *P. syringae*. In *eds1* and *pad4*, induction of *EDS5*, after infection with either virulent or avirulent *P. syringae* is blocked, places EDS1 and PAD4 upstream of EDS5 in the regulation of SA synthesis (Nawrath et al. 2002). Since EDS1 and PAD4 are required for resistance conferred by the same subset of *R* genes (TIR-NB-LRR) and have been shown to physically interact in planta, they are likely to function in the same pathway (Feys et al. 2001). However, the *eds1* mutation significantly impedes the onset of HR and confers full susceptibility, whereas *pad4* plants retain HR and show only intermediate susceptibility. Enhancement of the SA signal also occurs through a signal amplification loop involving ROS (Shirasu et al. 1997). The observation that SA binds the H2O2 scavenging enzymes catalase and ascorbate peroxidase (APX) and inhibits their activity led to the proposal that increases in H2O2 were responsible for signal transduction leading to *PR* gene induction and resistance (Chen et al. 1993; Durner and Klessig 1995). However, the concentrations of SA required for inhibition of catalase and APX are higher than those seen in systemic tissues after infection. In addition to the signal amplification loops described above, there is evidence for negative feedback of SA synthesis. In the SA-insensitive *npr1* mutant, levels of *ICS1* mRNA and SA are both elevated after infection compared to wild type (Delaney et al. 1995, Shah et al. 1994, Wildermuth et al. 2001). Furthermore, *npr1* mutants show reduced tolerance to exogenous SA (0.5 mM), failing to develop beyond the cotyledon stage (Cao et al. 1997, Kinkema et al. 2000).

NPR1-DEPENDENT SA SIGNALING

To identify components involved in SA signal transduction, a number of mutant screens were performed that identified multiple alleles of a single gene, *NPR1/NIM1* (Cao et al. 1994, Delaney et al. 1995; Glazebrook et al. 1996; Shah et al. 1997). Further characterization showed that the role of NPR1 is not limited to SAR. The *npr1* mutant

also displays enhanced disease symptoms when infected with virulent pathogens and is impaired in some *R* gene-mediated resistance, suggesting that NPR1 is important for restricting the growth of pathogens at the site of infection (Cao et al. 1994; Delaney et al. 1995; Glazebrook et al. 1996; Shah et al. 1997). NPR1 is required for another induced resistance response, known as induced systemic resistance (ISR), which is triggered by nonpathogenic root-colonizing bacteria and confers resistance to bacteria and fungi in aerial parts of the plant (Pieterse et al. 1996; Pieterse et al. 1998). *NPR1* is expressed throughout the plant at low levels and its mRNA levels rise two- to threefold after pathogen infection or treatment with SA (Cao et al. 1997; Ryals et al. 1997). *NPR1* expression is likely mediated by WRKY transcription factors as mutation of the WRKY binding sites (W-boxes) in the *NPR1* promoter abolished its expression (Yu et al. 2001).Overexpression of *NPR1* in *Arabidopsis* enhances resistance to *P. parasitica*, *P. syringae*, and *Erysiphe cichoracearum* with no apparent detrimental effects on the plant (Cao et al. 1998, Friedrich et al. 2001). The NPR1 protein has two protein-protein interaction domains, an ankyrinrepeat and a BTB/POZ (*Broad-Complex*, *Tramtrack*, *Bric-a-brac*/Poxvirus, Zinc finger) domain, as well as a putative nuclear localization signal and phosphorylation sites (Cao et al. 1997; Ryals et al. 1997). Functional studies have shown that accumulation of NPR1 in the nucleus after treatment with SAR inducers is essential for *PR* gene induction (Kinkema et al. 2000).

TGA Transcription Factors

The absence of any obvious DNA-binding domain and the presence of protein protein interaction domains in NPR1 prompted several laboratories to carry out yeast two-hybrid screens for NPR1-interacting proteins. In one of these screens, three small structurally similar proteins named NIMIN1, NIMIN2, and NIMIN3 (NIM interactor) were identified. NIMIN1 and NIMIN2 interact with the C terminus of NPR1, while NIMIN3 interacts with the N terminus (Weigel et al. 2001). The predominant NPR1 interactors found in the yeast two-hybrid screens were members of the TGA family of basic leucine zipper transcription factors. NPR1 interacts with the *Arabidopsis* TGA factors, TGA2, TGA3, TGA5, TGA6, and TGA7 but only weakly or not at all with TGA1 and TGA4 (Despr´es et al. 2000, Kim and Delaney 2002, Zhang et al. 1999, Zhou et al. 2000). TGA factors bind to activator sequence-1 (*as-1*) or *as-1*-like promoter elements (Katagiri et al. 1989), which have been found in several plant promoters activated during defense, including *Arabidopsis PR-1* (Lebel et al. 1998). Linker scanning mutagenesis of the *PR-1* promoter identified two *as-1*-like elements, *LS7* and *LS5*. *LS7* is a positive regulatory element required for induction by INA, whereas *LS5* is a weak negative regulatory element (Lebel et al. 1998). Despr´es *et al*, used these *cis*-elements as probes for electrophoretic mobility shift assays (EMSA) and showed that both TGA2 and TGA4 could bind to *LS7*, whereas only TGA2 could bind to *LS5* (Despr´es et al. 2000). Although NPR1 is clearly a positive regulator of *PR* genes, it may exert its function by either enhancing a transcriptional activator or inhibiting a transcriptional repressor. The presence of multiple *as-1*-like elements in the *PR-1* promoter and the differential binding affinities of each TGA factor to these elements as well as to NPR1 highlight the complexity of the regulatory mechanism. Indeed, in an EMSA performed by Despr´es *et al*, binding to the *as-1* element from the *35S* promoter was significantly enhanced in protein extracts from SA-treated plants (Despr´es et al. 2000). Another approach to study the role of TGA factors in vivo is to examine the phenotypes of mutant plants. As there are 10 TGA factors in *Arabidopsis* (Jakoby et al. 2002), functional redundancy may prevent observation of a mutant phenotype. Indeed, analysis of single knockout mutants of TGA2 and TGA3 revealed little phenotype (M. Kesarwani & X. Dong, unpublished observations). Consistent with this, overexpression or silencing of TGA2 did not alter resistance to a virulent strain of *P. parasitica* (Kim and Delaney 2002). However, overexpression of TGA5 enhanced resistance to *P. parasitica*, but this was not dependent on SA or NPR1 and did not correlate with *PR* gene expression. Using a reverse genetics approach, Li *et al*. isolated a knockout of the adjacent *TGA2* and *TGA5* genes (Li et al. 2001). This was crossed to a knockout of *TGA6* to create the *tga2 tga5 tga6* triple mutant (Zhang et al. 2003), thus deleting all members of one of three subclasses of TGA factors (Xiang et al. 1997). The *tga2 tga5 tga6* triple mutant has phenotypes similar to *npr1*, showing compromised SAR and decreased tolerance to high concentrations of SA. All three genes must be deleted to observe this phenotype, leading to the conclusion that TGA2, TGA5, and TGA6 are essential for and play redundant roles in the induction of SAR. Interestingly, the triple knockout and also the *tga2 tga5* double mutant have increased *PR-1* expression in the absence of SAR induction, suggesting that TGA factors also play a role in the repression of basal *PR-1* expression. This might be through interaction with the negative *LS5* element in the *PR-1* promoter (Lebel et al. 1998). As an alternative to mutant analysis, dominant-negative versions of TGA factors that can no longer bind to DNA were expressed in tobacco and *Arabidopsis*. In tobacco, overexpression of a dominant-negative *TGA2.2* decreased *as-1*-binding activity and *PR* gene induction (Niggeweg et al. 2000). In another study,

a dominant-negative version of *Arabidopsis* TGA2 was expressed in tobacco (Pontier et al. 2001). To observe activity of specific TGA factors in vivo, chimeric transcription factors have been constructed in which TGA2 or TGA3 were fused to the yeast GAL4 DNA-binding domain. Fan & Dong showed that replacing the bZIP domain of TGA2 with the GAL4DNA-binding domain produced a transcription factor that activated the expression of a *UASGAL4: GUS* reporter construct in response to INA or SA (Fan and Dong 2002). Johnson et al. used a similar heterologous system to show that TGA3 is also a transcriptional activator (Johnson et al. 2003).

Redox Signaling

The in vivo interaction of NPR1 with TGA factors requires induction with SA, even though both proteins are constitutively expressed. Until recently, the controlling mechanisms for NPR1 nuclear localization and activation of TGA factors were unclear. Two exciting new papers have revealed that changes in the redox status of the cell after SA treatment play an important role in this regulation (Despr´es et al. 2003; Mou et al. 2003). The observation that NPR1-like proteins from different species contain ten conserved cysteines suggested that NPR1 might be under redox-regulation. tested this hypothesis by examining NPR1 under different redox conditions (Mou et al. 2003). As discussed earlier, in yeast two-hybrid studies NPR1 interacts strongly with TGA2 and TGA3 but very weakly or not at all with TGA1 and TGA4 (Despr´es et al. 2000; Zhou et al. 2000). Using a plant two-hybrid assay in *Arabidopsis*, Despr´es et al. demonstrated a physical interaction between NPR1 and TGA1 (Despr´es et al. 2003). Using domain swapping between TGA1 and TGA2, the plant-specific regulatory regionwas defined to a 30 aa region containing two cysteine residues in TGA1 (and TGA4) that are not found in TGA2 or other TGA factors. Mutation of these residues in TGA1 allowed interaction with NPR1 in yeast and in untreated leaves. A clever labeling experiment designed to distinguish between reduced and oxidized cysteine residues showed that TGA1 (and/or TGA4) exists in both oxidized and reduced forms in untreated leaves. After SA treatment, only the reduced form was detected (Despr´es et al. 2003).

CONCLUSIONS

Our understanding of SAR has increased considerably over recent years as we have begun to elucidate the molecular mechanisms underlying this response. Many of the processes contributing to SAR are clearly required in both local and systemic tissues and contribute to basal disease resistance. These include the synthesis of SA, changes in redox status, and the induction of defense gene expression. Systemic acquired resistance is a general and rather elegant response developed by plants against various invaders. A substantial body of knowledge has accumulated since the early descriptions of the phenomenon, and observations now extend to the molecular events underlying SAR. Knowledge of SAR promises to be useful in developing new strategies for crop protection. New chemical inducers of resistance have already been developed commercially with potential application in the cereal market. By analogy to human medicine where in an emergency vaccination can be complemented by antibiotics, crop protection with immunizing chemicals is conceivable with limited input of pesticides. Induction of SAR to control infection of crop plants is already being used in the field by application of BTH and it has been suggested that NPR1 overexpression is another viable strategy. Better understanding of the SAR signaling pathway will certainly lead to new environmentally friendly methods of crop protection.

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