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Salicylic Acid and Systemic Acquired Resistance to Pathogen Attack

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New insights into the phenomenon of systemic acquired resistance have been gained in recent years, by the use of techniques in molecular genetics and biology that have replaced the largely descriptive approach of earlier work. The isolation of mutants in the signal transduction pathway from induction to expression of resistance as well as the use of transgenic plants over-expressing or suppressing the expression of putative candidate genes involved in systemic acquired resistance and its signalling have identified several steps in the establishment of plant resistance. In this review the latest developments implicating salicylic acid as a signal molecule in systemic resistance are discussed and contrasted with new signalling pathways which, seemingly, are based on alternative mechanisms.

Key words: *Arabidopsis thaliana*, induced systemic resistance (ISR), hypersensitive reaction, plant defence, oxidative burst, systemic acquired resistance (SAR), signal transduction.

INTRODUCTION

The natural resistance of plants to pathogens and herbivorous insects is based on the combined effects of preformed barriers and induced mechanisms. In both cases, plants use physical and antimicrobial defences against the invaders. In contrast to constitutive resistance, induced resistance relies on recognition of an invader and subsequent signal transduction events leading to the activation of defences. In many cases, localized infection by pathogens induces resistance directed at a broad spectrum of widely different pathogens such as fungi, bacteria or viruses. This resistance is expressed locally at the site of pathogen attack and systemically, in uninfected parts of the plant. The defence mechanisms involved include a combination of physical changes such as cell wall lignification, papilla formation or the induction of various pathogenesis-related proteins (PRs) (reviewed in Kessmann *et al.*, 1994; Schneider *et al.*, 1996; Sticher, Mauch-Mani and Métraux, 1997; Van Loon, 1997). Systemic acquired resistance implies the production by the plant of one or several translocated signals that are involved in the activation of resistance mechanisms in uninfected parts. Thus, a first infection predisposes the plant to resist further attacks. This latter aspect makes it comparable to immunization in animals and humans. Several recent reviews have dealt with various aspects of systemic acquired resistance (Kessmann *et al.*, 1994; Hunt and Ryals, 1996; Ryals *et al.*, 1996; Schneider *et al.*, 1996; Delaney, 1997; Sticher *et al.*, 1997; Van Loon, 1997; Yang, Shah and Klessig, 1997). Evidence has accumulated that salicylic acid (SA) is a signal for systemic resistance (reviewed in Klessig and Malamy, 1994; Ryals *et al.*, 1996; Schneider *et al.*, 1996; Sticher *et al.*, 1997; Yang *et al.*, 1997). Recently, a number of reports have indicated that plant growth promoting rhizobacteria (PGPR) can induce systemic acquired resistance that operates independently of SA (Pieterse *et al.*, 1996). The nature of the systemic signal involved in PGPR-induced resistance is not known, but it does not require SA (Pieterse *et al.*, 1996). To distinguish systemic SA-dependent defences resulting from pathogen pretreatments (or pretreatments with SA or SA-like compounds) from other systemic responses that operate without SA, Kloepper and recently Van Loon proposed to term the former reactions systemic acquired resistance (SAR) and the latter reactions induced systemic resistance (ISR). This second term encompasses all systemic defence reactions (Kloepper, Tuzun and Kuc, 1992; Van Loon, 1997). The goal of the present review is to provide an update on SAR and ISR.

SYSTEMIC ACQUIRED RESISTANCE (SAR)

In the early 1990s, a possible link between the accumulation of SA and SAR was discovered (Malamy *et al.*, 1990; Métraux *et al.*, 1990; Rasmussen, Hammerschmidt and Zook, 1991). Together with earlier observations (White, 1979) this opened the field to numerous investigations on molecular events involved in signalling of induced resistance. The importance of SA in SAR induction was documented by subsequent experiments with transgenic plants over-expressing a salicylate hydroxylase gene (the *nahG* gene) (Gaffney *et al.*, 1993; Delaney *et al.*, 1994). Grafting experiments in tobacco between *nahG* and wild type plants, as well as leaf excision experiments in cucumber, supported the notion that SA is not the primary mobile signal exported from the infected leaf to other parts of the plant (Rasmussen *et al.*, 1991; Vernooij *et al.*, 1994). In contrast, other experiments have shown that up to 70% of SA accumulating in upper, non-infected leaves of infected tobacco plants results from SA transport from the infected leaf (Shulaev,

Leon and Raskin, 1995). Labelling experiments in cucumber also indicate that SA is mobile during SAR (Mölders, Buchala and Metraux, 1996). Further experiments in cucumber have shown that SA as well as 4-hydroxy benzoic acid (4HBA) increase in phloem sap concomitantly with an increase in activity of phenylalanine ammonia-lyase in the petiole. It was proposed that SA and 4HBA are produced *de novo* in stems and petioles in response to a mobile signal from the leaf lamina (Smith-Becker *et al.*, 1998). In tobacco, volatile methyl salicylate (MeSA) is produced from SA after infection and can induce defence responses by conversion to SA (Shulaev, Silverman and Raskin, 1997). MeSA levels in plant tissue also parallel the increase in SA concentration locally and systemically after viral or bacterial infections (Seskar, Shulaev and Raskin, 1998). MeSA represents an inactive precursor of SA that can be translocated and converted to SA. Indeed, plants expressing the *nahG* gene are unable to respond to MeSA indicating that this compound has no direct effect on the induction of disease responses (Seskar *et al.*, 1998). MeSA might supplement the effect of SA for intraplant signalling and it might be of relevance for plant to plant communication (Shulaev *et al.*, 1997), although more evidence is still necessary to support the last point. In summary, most studies agree that SA acts as an endogenous signal involved in defence signalling. Whether SA is itself the primary systemic signal exported or merely transported along with the primary systemic signal remains a matter of debate. Future work should be directed at elucidating the nature of the primary systemic signal.

A mode of action of SA was proposed based on the finding that SA binds to, and inhibits, catalase (Chen, Silva and Klessig, 1993). Catalase inhibition would lead to an increase in the concentration of hydrogen peroxide (H_2O_2) or active oxygen species derived from it that arise during respiration, photosynthesis, photorespiration or during the hypersensitive response against pathogens. H_2O_2 could have a direct antibiotic activity against invading pathogens. H_2O_2 and its derivatives could also act as intermediates in the signalling cascade for the expression of genes related to defence (Chen *et al.*, 1993; Durner, Shah and Klessig, 1997). In agreement with this hypothesis, resistance-inducing compounds such as INA (2,6-dichloroisonicotinic acid) and BTH (benzo-(1,2,3)-thiadiazole-7-carbothioic acid *S*-methyl ester) (Métraux *et al.*, 1991; Friedrich *et al.*, 1996) were shown to have similar effects on catalase (Conrath *et al.*, 1995). The catalase inhibition hypothesis was seriously questioned by several studies that showed that: (1) the induction of defence-related proteins such as PR-1 does not result from H_2O_2 derived from SA-inhibited catalase but rather from SA directly; (2) catalase activity is not decreased after pathogen inoculation or SA treatment; (3) SA levels in systemic tissue are too small to inhibit catalase; and (4) H_2O_2 at high levels can induce the production of SA (Bi *et al.*, 1995; Leon, Lawton and Raskin, 1995; Neuenschwander *et al.*, 1995; Summermatter *et al.*, 1995). The importance of catalase inhibition by SA was further tested using transgenic tobacco plants with diminished catalase activity resulting from antisense expression of the catalase gene. No increase in the constitutive production of defence related PR-1 protein was observed (Chamnongpol *et al.*, 1996). Three transgenic lines that exhibited the most severe inhibition in catalase activity developed high levels of PR-1 expression and showed enhanced resistance to TMV (Takahashi *et al.*, 1997). The progeny of crosses of these lines and *nahG* plants developed necroses like the parent plants but did not express constitutively PR-1 nor develop enhanced resistance, indicating that SA is required for the induction of defence responses in catalase deficient plants (Du and Klessig, 1997*a*). Similarly, SA is needed for hypersensitive cell death in bacteria-infected soybean cell cultures. In this system, SA does not inhibit catalase and ascorbate peroxidase nor increase the metabolism of H_2O_2 (Tenhaken and Rübel, 1997). Summarizing, catalase inhibition and the subsequent rise of H_2O_2 levels is unlikely to be the main mode of action of SA in the induction of defence responses.

A further mode of action for SA was proposed by virtue of its ability to form SA free radicals upon inhibition of heme-containing enzymes such as peroxidase or catalase (Durner *et al.*, 1997). It was suggested that such phenolic free radicals are potent initiators of lipid peroxidation, the products of which might activate defence reactions (reviewed by Goodman and Novacky, 1994). It remains to be shown that lipid peroxidation products accumulate to high enough levels and soon enough after infection to serve as effective inducers of defence responses.

SA is an endogenous trigger of thermogenesis and increases the expression of an alternative oxidase linked to this generation of heat (Raskin and Meeuse, 1987; Rhoads and MacIntosh, 1992). SA-dependent induction of alternative oxidase might be involved in maintaining the NADPH-requiring oxidative burst after pathogen infection that is assumed to lead to the activation of defence responses. The alternative oxidase participates in the oxidation of pyruvate produced from the pentose-phosphate pathway that generates NADPH (Lennon *et al.*, 1997). More work is required to assess the relevance of SA-induced alternative oxidase in the control of adenylate levels generated at the site of infection.

Recently, a 25 kD soluble SA-binding protein was identified in tobacco leaves with a reversible affinity for SA (k_d = 90 nM) that is 150-fold higher than that for catalase (Du and Klessig, 1997*b*). Since SA can chelate iron, it would be interesting to know if this binding protein is a metalloprotein like catalase. The role of this SA-binding protein in the action of SA has now to be evaluated further.

Several studies report that SA pretreatment enhances the subsequent response to elicitor treatment (Kauss *et al.*, 1992, 1994; Draper, 1997). This suggests that, in addition to being a direct intermediate necessary for the induction of PRs, SA also has an effect on the very early responses that lead to the oxidative burst, cell death and its own synthesis. Indeed, a standing question is whether the low levels of SA present in unchallenged leaves of previously challenged plants can have an effect on the timing or extent of the oxidative burst in response to challenge infection (conditioning effect). Recent data indicate that low levels of SA, that is 50–200 μM, can indeed enhance the initial oxidative burst and ensuing cell death after exposure to avirulent bacteria (Shirasu *et al.*, 1997). It would now be interesting to

know if such observations apply to whole leaves of intact plants. A report by Mur *et al.* (1996) on TMV-infected tobacco plants encourages this possibility. The biochemical basis of SA-induced conditioning remains to be elucidated.

SA can induce the expression of a number of defence-related genes and proteins (Hunt and Ryals, 1996; Schneider *et al.*, 1996; Sticher *et al.*, 1997; van Loon, 1997; Yang *et al.*, 1997). An increase in enzyme activities associated with antioxidative processes was observed in non-infected leaves of TMV-infected tobacco (Fodor *et al.*, 1997). This might perhaps lead to decreased lesions observed after infection by the challenge pathogen. A somewhat similar suggestion was made for systemically protected arabidopsis leaves (Summermatter *et al.*, 1995, 1996). This might enlarge the spectrum of activities induced after a local infection not only to include principles that act directly against the invader but also to self-protection against the stress inflicted during invasion.

The activation of plant defence pathways will eventually culminate in the activation of defence genes by trans-activation of pathogen- or SA-responsive elements on their promoters (see review by Yang *et al.*, 1997). Several SA-inducible promoters contain as-1 elements that bind to bZIP proteins, and their activation seems not to depend on synthesis of new proteins but rather on the activation of SA-stimulated phosphorylation of a pre-existing factor (Qin *et al.*, 1994; Jupin and Chua, 1996). Since SA and pathogens like TMV were shown to activate a MAP kinase, it is tempting to speculate that this enzyme is involved in the activation of the as-1 element binding protein (Zhang and Klessig, 1997). Other SA-inducible genes such as PR-1a have promoters equipped with several Myb binding sites. Myb proteins are transcription factors regulated by the redox state of the cell, and the expression of the *myb1* gene in tobacco was shown to be induced rapidly by SA (Yang and Klessig, 1996). Possibly this protein as well as others, such as the GT-like protein (Buchel *et al.*, 1996), could act alone or in combination to modulate the expression of pathogen- or SA-treated tissue.

Since SAR also operates in arabidopsis (Cameron, Dixon Lamb, 1994; Mauch-Mani and Slusarenko, 1994; Summermatter *et al.*, 1995), a powerful genetic system became available to approach the mechanisms leading to SAR by mutational analysis (Delaney, 1997; Durner *et al.*, 1997; Yang *et al.*, 1997). The recessive mutants *npr1* (no *PR*-1), *sai1* (*s*alicylic *a*cid *i*nsensitive), *nim1* (*no-im*munity), *eds5* (*e*nhanced *d*isease *s*ymptoms) all show normal necrotic hypersensitive-type lesions (HR) when inoculated with an avirulent pathogen but are compromised in their defence responses (Cao *et al.*, 1994, 1997; Delaney, Friedrich and Ryals, 1995; Ryals *et al.*, 1996; Shah, Tsui and Klessig, 1997). The allelic mutants *npr1*, *sai1* and *nim* appear to produce normal SA levels but are blocked downstream of its perception. Chemical inducers such as INA or BTH have no effects in these mutants indicating that their action involves a similar signal transduction pathway. The *npr* 1 gene encodes a 60 kD protein containing several ankyrin repeats (Cao *et al.*, 1997). This may indicate that Npr1 interacts with other proteins in the SA transduction pathway.

Other mutants such as *acd2* (*a*ccelerated *c*ell *d*eath), *lsd* (*l*esion *s*imulating *d*isease), *cpr1* and *cpr5* (*c*onstitutive *PR*), *cep1* (*c*onstitutive *e*xpression of *P*R) or *cim3* (*c*onstitutive *im*munity) show a constitutive activation of defence and accumulate high levels of SA (Bowling *et al.*, 1994, 1997; Dietrich *et al.*, 1994; Greenberg *et al.*, 1994; Weymann *et al.*, 1995; Ryals *et al.*, 1996). In these cases SA levels are higher than normal and often the mutations are accompanied by spontaneous lesion formation. Thus, it is not always clear if these mutations reflect a mutation in the defence signalling pathway, in genes involved in SA biosynthesis or in some other metabolic pathway leading to increased stress with accompanying SA accumulation. Homozygous double mutants for *cpr5* and *npr1* or *cpr5* and *nahG* are susceptible to the virulent bacterium *Pseudomonas syringae* pv *maculicola* ES4326 and do not express PR-1 indicating that *cpr5* acts upstream of SA (Bowling *et al.*, 1997). However, *cpr5*, *npr1* plants remain resistant to the fungal pathogen *Peronospora parasitica* Noco2 and exhibit elevated expression of a plant defensin (PDF.2). This means that *cpr5* mutants express resistance that is mediated by both an *npr1*-dependent and an *npr1*-independent SAR pathway. Thus, SAR might well consist of more than one signalling pathway. The next section will review further evidence supporting this possibility.

INDUCED SYSTEMIC RESISTANCE (ISR)

Plant growth promoting rhizobacteria (PGPRs), belonging mainly to the group of fluorescent *Pseudomonas* spp, have been known to control plant diseases by suppressing pathogen development through antibiotic effects or by competing for iron through siderophores. Investigations into the mechanisms of resistance induction by PGPRs revealed that these bacteria also confer systemic protection against pathogenic organisms by inducing defensive capabilities in the plant. To demonstrate this, the inducing bacteria and the challenging organisms were spacially separated from each other to rule out any direct antibiotic effects (Van Peer, Niemann and Schnippers, 1991; Pieterse *et al.*, 1996). ISR due to *P. fluorescens* has been demonstrated in several species including carnation (Van Peer *et al.*, 1991), radish (Leeman *et al.*, 1995), arabidopsis (Pieterse *et al.*, 1996), cucumber (Wei, Kloepper and Tuzun, 1991) and tobacco (Maurhofer *et al.*, 1995). Experiments using bacterial lipopolysaccharides (Leeman *et al.*, 1995) or heat-killed bacteria (Van Peer and Schippers, 1992) to induce ISR support the thesis that the observed resistance is due to an enhancement of the plant defence response and not to translocation of toxic bacterial metabolites to the site of infection.

Recent findings indicate that SAR and ISR might confer resistance through different pathways. Whereas SAR leads to an increase in SA and accumulation of PR proteins, Pieterse *et al.* (1996) showed that in arabidopsis ISR mediated by strain WCS417 of *P. fluorescens* is independent from SA accumulation and PR gene expression, since ISR takes place in plants expressing the *nahG* gene.

The existence of additional or alternate pathways to induce resistance in plants is also supported by investigations

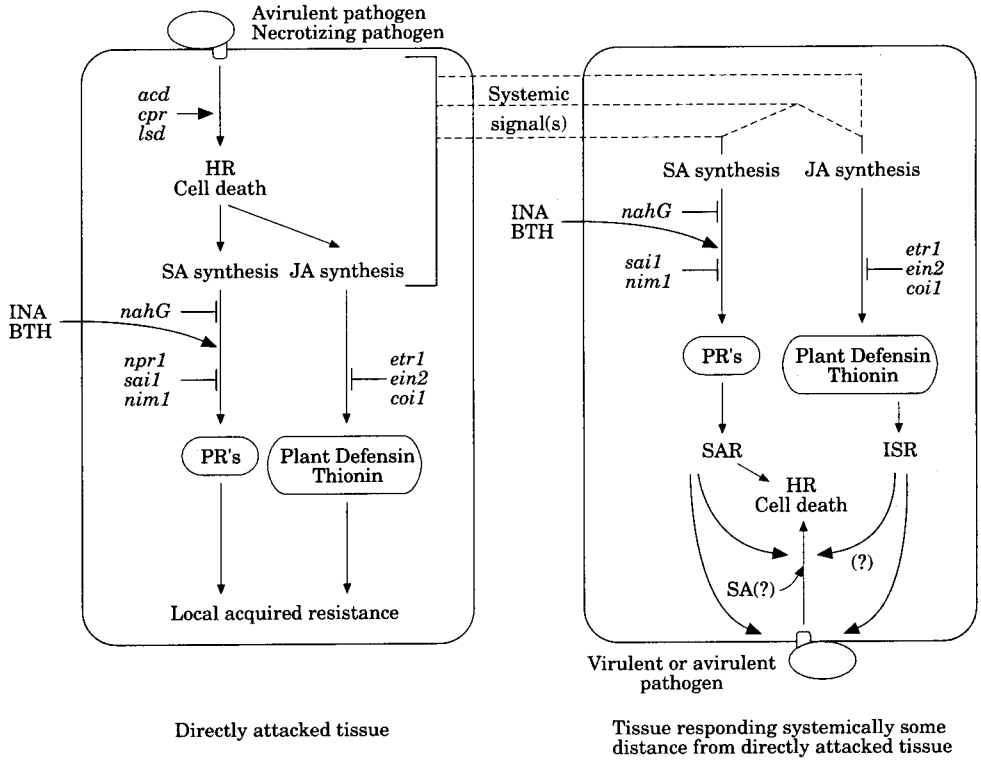

FIG. 1. Diagrammatic representation of the induction and expression of systemic acquired resistance. The scheme shows what happens after a first attack by a virulent or necrotizing pathogen. It also shows the systemic responses taking place before and after a challenge infection in the systemic tissue. BTH, Benzo-(1,2,3)-thiadiazole-7-carbothioic acid *S*-methyl ester; HR, hypersensitive reaction; INA, 2,6-dichloroisonicotinic acid; ISR, induced systemic reaction; JA, jasmonic acid; PR, pathogenesis-related proteins; SA, salicylic acid; SAR, systemic acquired resistance.

of small, cystein-rich plant defensins (PDFs) and thionins during defence reactions. Using an arabidopsis model system, Epple, Apel and Bohlmann (1995) demonstrated that the thionin 2.1 gene (*Thi2.1*) is inducible by methyl jasmonate, silve nitrate and pathogenic fungi but not by SA or ethephon (simulating ethylene treatment). These results suggest that thionin induction depends on a signal transduction pathway other than PR induction. Also, using an arabidopsis system, the group of Broekaert (Penninckx *et al.*, 1996) showed that the antifungal plant defensin *PDF1.2* is highly induced by inoculation with an avirulent isolate of *Alternaria brassicola* and accumulates in the leaves after treatment with methyl jasmonate, ethylene, paraquat and rose bengal, whereas none of these chemicals leads to an accumulation of the *PR-1* mRNA. INA and SA, in contrast, induce the accumulation of *PR-1* mRNA but neither the plant defensin protein nor its mRNA. In arabidopsis plants expressing the *nahG* gene (Delaney *et al.*, 1994) and in the *npr1* mutant (Cao *et al.*, 1994), induction with an avirulent fungus still leads to the accumulation of defensin. The arabidopsis mutants *coi1* (Feys *et al.*, 1994) and *ein2* (Guzman and Ecker, 1990), blocked in their response to methyl jasmonate and ethylene, respectively, are still able to induce PR-1 but have a highly reduced capacity to accumulate the plant defensin after fungal induction treatment. In the accelerated cell death mutant *acd2* (Greenberg *et al.*, 1994) of arabidopsis both PR-1 and plant defensin transcript levels are constitutively elevated.

In tobacco, treatment with culture filtrates from the plant pathogenic bacterium *Erwinia carotovora* subsp. *carotovora* leads to local as well as systemic induction of resistance against the same organism (Vidal *et al.*, 1988). Testing of individual enzymes from the culture filtrate for their ability to induce resistance and the expression of the defence related basic β-1,3-glucanase gene revealed that the induction was mainly due to the activity of pectic enzymes and cellulase (β-1,4-glucanase). Most interestingly, SA does not seem to be involved in this resistance induction process, since the authors were able to demonstrate that in *nahG* plants resistance still can be induced as described above (Vidal *et al.*, 1997). A similar phenomenon was observed with the rhizobacterial strain of *Serratia marcescens* 90-166 (Press *et al.*, 1997). This strain itself produces SA but the observed induction of resistance against *Pseudomonas syringae* pv. *tabaci* in *nahG* tobacco should not be caused by SA since it is continuously converted to catechol in these plants.

CONCLUSIONS

Insights have been gained by studying the general process of induced resistance to pathogen attack using both mutational and physiological approaches. There is now good evidence

that both SA-dependent and SA-independent pathways are involved in systemic signalling for defence responses (Fig. 1). It remains to be seen which parts of the signalling pathways are common to both. The nature of the induced responses and their targets, pathogens, or perhaps their induction by other stresses, especially with regard to the SA-independent pathway are intriguing questions for future investigations.

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