Cross-talk between signaling pathways leading to defense against pathogens and insects

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In nature, plants interact with a wide range of organisms, some of which are harmful (*e.g.* pathogens, herbivorous insects), while others are beneficial (*e.g.* growth-promoting rhizobacteria, mycorrhizal fungi, and predatory enemies of herbivores). During the evolutionary arms race between plants and their attackers, primary and secondary immune responses evolved to recognize common or highly specialized features of microbial pathogens (Chisholm et al., 2006), resulting in sophisticated mechanisms of defense. Although the arms race between plants and herbivorous insects has been intensively debated (Schoonhoven et al., 2005), knowledge of the underlying mechanisms is relatively limited. In the past years, various genomics approaches exponentially expanded our understanding of the molecular mechanisms by which plants tailor their defense response to pathogens, insects and beneficials. The plant hormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) emerged as key players in the regulation of the signaling networks involved (Howe, 2005; Pozo et al., 2005; Grant and Lamb, 2006; Van Loon et al., 2006; Von Dahl and Baldwin, 2007). SA-, JA-, and ET-dependent pathways regulate defense responses that are differentially effective against specific types of attackers. Pathogens with a biotrophic lifestyle are generally more sensitive to SA-dependent responses, whereas necrotrophic pathogens and herbivorous insects are commonly deterred by JA/ET-dependent defenses (Kessler and Baldwin, 2002; Glazebrook, 2005). In beneficial interactions, JA and other oxylipins appear to play a dominant

role in the response of the plant as well (Van Poecke and Dicke, 2004; Hause and Fester, 2005; Pozo et al., 2005).

Clearly, the defense signaling pathways that are activated during the interaction of plants with herbivores, pathogens and beneficials show a high degree of overlap, suggesting that activation of the respective defense responses leads to cross resistance. Indeed, activation of JA/ET-dependent induced systemic resistance (ISR) in *Arabidopsis* by beneficial *Pseudomonas fluorescens* rhizobacteria results in enhanced protection against predominantly necrotrophic pathogens, whereas induction of SA-dependent systemic acquired resistance (SAR) by necrotizing pathogens is primarily effective against biotrophic pathogens (Ton et al., 2002). Furthermore, caterpillars of the herbivore *Pieris rapae*, which stimulate the production of JA and ET in *Arabidopsis*, trigger a defense response that not only affects insect performance, it also provides enhanced protection against the microbial pathogens *Pseudomonas syringae, Xanthomonas campestris*, and Turnip crinkle virus (TCV) (De Vos et al., 2006).

Pathway cross-talk to fine-tune defense

A major focus in plant defense signaling research is to uncover key mechanisms by which plants tailor their response to parasites and mutualists, and to investigate how plants cope with simultaneous interactions with multiple aggressors. A first line of regulation is obviously the great regulatory potential of the so-called signal signature. The production of the defense signals SA, JA, and ET varies greatly depending on the type of organism that interacts with the plant. The quantity, composition and timing of this signal signature results in the activation of a specific set of genes that eventually shapes the outcome of the defense response that is triggered by the attacker encountered (De Vos et al., 2005; Mur et al., 2006). However, additional levels of regulation are important as well. For example, the bacterial pathogen *P. syringae,* the necrotrophic fungus *Alternaria brassicicola,* the cell-content feeding thrips *Frankliniella occidentalis* and the chewing caterpillar *P. rapae* all stimulate JA biosynthesis and JA-responsive gene expression in *Arabidopsis* (De Vos et al., 2005). Yet, the majority of the JA-responsive genes that are activated by each attacker are specific for the plant-attacker combination. Hence, JA plays an important primary role in the orchestration of the plant's defense response, but other regulatory mechanisms, such as pathway cross-talk, or additional attacker-induced signals, eventually shape the highly complex attacker-specific defense response.

There is ample evidence that SA, JA, and ET pathways interact, either positively or negatively (Beckers and Spoel, 2006). This pathway cross-talk is thought to provide the plant with a powerful regulatory potential that helps deciding which defensive strategy to follow, depending on the type of attacker encountered (Reymond and Farmer, 1998). In *Arabidopsis*, pharmacological experiments showed that SA strongly antagonizes methyl jasmonate (MeJA)-induced expression of JA-responsive marker genes, such as *PDF1.2, LOX2* and *VSP2* (Spoel et al., 2003). This antagonistic effect of SA on JA signaling was also observed when the JA pathway was biologically activated by the necrotrophic pathogens *Botrytis cinerea* and *A. brassicicola,* or the insect herbivores *P. rapae* and *F. occidentalis*, and when the SA and JA pathways was triggered simultaneously by the biotrophic pathogen *Hyaloperonospora parasitica* and the herbivore *P. rapae,* respectively. These results indicate that that pathogen-induced SA can negatively affect JA signaling and that during multitrophic interactions, the SA pathway can be prioritized over the JA pathway.

Decoy of plant defenses

It appears that attackers have also evolved ways to manipulate plants for their own benefit by suppressing induced defenses via modulation of the plant's signaling network. A nice example is the response of *Arabidopsis* to silverleaf whitefly (*Bemisia tabaci*) nymphs. The nymphs of this phloem-feeding insect sabotage effectual JA-dependent host defenses by activating the antagonistic SA signaling pathway (Zarate et al., 2007). Pathogens suppress host defenses as well, by using virulence factors that antagonize the plant's immune response (Nomura et al., 2005). One of these virulence factors is the *P. syringae* phytotoxin coronatine, which functions as a JA analog. During the interaction with susceptible *Arabidopsis* plants, coronatine suppresses SA-dependent defenses, thereby promoting susceptibility to this pathogen (Zhao et al., 2003; Brooks et al., 2005; Cui et al., 2005).

Molecular players in SA/JA cross-talk

Antagonism between SA and JA signaling emerged as the most prominent of all signal interactions studied to date (Dong, 2004; Pieterse and Van Loon, 2004; Bostock, 2005; Nomura et al., 2005). However, the underlying molecular mechanisms of SA/JA cross-talk are to a large extent unknown. Several key regulatory proteins involved in SA/JA cross-talk have been identified in *Arabidopsis*. For instance, the transcription factor WRKY70 was

shown to act as an activator of SA-responsive genes and a repressor of JA-inducible genes, thereby functioning as a molecular switch between both pathways (Li et al., 2004). Previously, we demonstrated that the defense regulatory protein NPR1 is required for SA/JA cross-talk (Spoel et al., 2003; Pieterse and Van Loon, 2004). Induction of the SA response, either by pathogen infection or by exogenous application of SA, strongly suppressed JA-responsive genes such as *PDF1.2, LOX2,* and *VSP2.* However, in mutant *npr1-1* plants, this SA-mediated suppression of JA-responsive gene expression was completely abolished. Nuclear localization of NPR1, which is essential for SA-mediated defense gene expression (Kinkema et al., 2000), was not required for the suppression of JA-responsive genes, indicating that SA/JA cross-talk is modulated through a function of NPR1 in the cytosol (Spoel et al., 2003; Pieterse and Van Loon, 2004). Recently, overexpression of the SA-regulated glutaredoxin GRX480 was found to antagonize JA-responsive *PDF1.2* transcription (Ndamukong et al., 2007), suggesting a role for redox regulaton in SA/JA cross-talk.

SA/JA cross-talk is conserved among *Arabidopsis* accessions

Naturally occurring variation in *Arabidopsis* accessions can be exploited to study the biological relevance and genetics of specific plant traits such as resistance to pathogens and pests (Koornneef et al., 2004). To investigate whether *Arabidopsis* displays natural variation for SA/JA cross-talk, we analyzed the antagonistic effect of SA on MeJA-induced *PDF1.2* transcription in 18 *Arabidopsis* accessions (An-1, Bur-0, C24, Col-0, Cvi-0, Di-0, Eri-1, Fei-0, Kond, Kyo-1, L*er*-0, Ll-0, Ren-0, RLD-1, Sha, Uk-4, Wei-0, Ws-2) collected from very different geographical origins. All accessions were treated with standard doses of SA (1 mM), MeJA (0.1 mM), or a combination of both chemicals. One day later, the expression of SA-responsive *PR-1* and JA-responsive *PDF1.2* was assessed. In the SA/MeJA combination treatments, SA-induced *PR-1* expression was not affected by MeJA in the majority of the accessions. Conversely, all accessions displayed a strong SA-mediated down-regulation of both MeJA-induced and basal levels of *PDF1.2* transcription. The relative suppression of *PDF1.2* transcription in the SA/JA treatment in comparison to the JA treatment ranged between 46% to 96%. These results demonstrate that SA-mediated antagonism on JA-responsive gene expression is conserved among *Arabidopsis* accessions, suggesting an important role of this phenomenon for plant survival.

SA/JA cross-talk acts downstream of octadecanoid biosynthesis

One of the targets of SA to suppress JA-responsive gene transcription is the octadecanoid biosynthesis pathway. Besides the JA-responsive marker genes *PDF1.2* and *VSP2,* genes encoding enzymes of the JA biosynthesis pathway, such as *LOX2, AOS*, *AOC3,* and *OPR3*, were also sensitive to SA-mediated suppression, suggesting that octadecanoid biosynthesis is indeed a potential target of SA in the suppression of the JA response. However, the octadecanoid biosynthesis null mutant *dde2* showed wild-type levels of SA-mediated cross-talk when the JA response was activated chemically by MeJA. These results demonstrate that SA targets the JA-signaling pathway at a position downstream of octadecanoid biosynthesis.

SA/JA cross-talk coincides with a cellular increase in glutathione levels

During plant-attacker interactions, the kinetics of SA and JA signaling are highly dynamic. Mimicking this dynamic response by applying SA and MeJA at different concentrations and time intervals revealed that *PDF1.2* transcription is readily suppressed when SA was applied at the onset of the JA response, and that this SA/JA antagonism is long-lasting. However, when SA was applied more than 30 h prior to MeJA, the suppressive effect of SA was completely absent, indicating that this antagonistic effect is transient. Changes in the cellular redox state play a major role in SA signal transduction (Mou et al., 2003). SA-mediated redox changes activate the regulatory protein NPR1 by monomerization of inactive NPR1 oligomers, resulting in the induction of SA-responsive genes such as *PR-1* (Mou et al., 2003; Dong, 2004). SA-activated NPR1 is also essential in mediating the antagonism between SA- and JA-dependent signaling (Spoel et al., 2003). Therefore, we hypothesized that the transient nature of the antagonistic effect of SA on JA signaling might be associated with changes in the cellular redox state. As a marker of the redox potential, we monitored the level of glutathione in *Arabidopsis* leaves upon application of SA. Glutathione is a low-molecular weight antioxidant that functions as a major determinant of cellular redox homeostasis. SA treatment resulted in a transient increase in the level of glutathione, which returned to baseline levels after 30 h. Interestingly, the change in redox potential coincided with the window of opportunity during which SA was able to suppress MeJA-induced *PDF1.2* transcription. Hence, we postulate that the antagonism between SA and JA signaling pathways is redox regulated.

SA/JA cross-talk: beneficial or deleterious?

In our pharmacological studies, we predominantly observed an antagonistic effect of SA on JA-responsive gene expression, while MeJA had virtually no effect on the SA-responsive marker gene *PR-1*. Early studies in tomato already revealed that SA and its acetylated form aspirin are potent suppressors of the JA-dependent wound response in tomato (Doherty et al., 1988; Peña-Cortés et al., 1993; Doares et al., 1995). Thus, activation of the SA pathway, such as upon infection by a necrotizing pathogen, may result in enhanced susceptibility to insect feeding. Indeed, trade-offs between SA-dependent pathogen resistance and JA-dependent defense against insect herbivory have been repeatedly reported (Pieterse et al., 2001; Bostock, 2005). In *Arabidopsis*, the SA pathway has been shown to inhibit JA-dependent resistance against tissue chewing herbivores such as *Spodoptera exigua* (beet armyworm) (Cipollini et al., 2004) and *Trichoplusia ni* (cabbage looper) (Cui et al., 2005), and necrotrophic pathogens such as *Alternaria brassicicola* (Kariola et al., 2005). Intriguingly, some herbivores have been demonstrated to induce the SA pathway to actively suppress effectual JA-dependent defenses and thereby escape host defense (Zarate et al., 2007). Hence, depending on the plant-attacker combination, the antagonistic effect of SA on JA-dependent defense responses may either be beneficial or deleterious.

Priming for enhanced defense

Priming for enhanced defense adds yet another layer of complexity to the way plants can adapt to their biotic environment. In primed plants, defense responses are not directly activated, but upon perception of biotic or abiotic stress signals, host defenses are accelerated resulting in an enhanced level of resistance (Conrath et al., 2006). The primed state can be induced biologically by beneficial rhizobacteria (Verhagen et al., 2004), mycorrhizal fungi (Pozo et al., 2005), pathogens (Cameron et al., 1999), and insect herbivores (Engelberth et al., 2004; De Vos et al., 2006), but also chemically, such as by low doses of SA (Mur et al., 1996), JA (Kauss et al., 1994) or β-aminobutyric acid (Ton et al., 2005). By studying the costs and benefits of priming in *Arabidopsis,* it was recently shown that the fitness costs of priming are substantially lower than those of constitutively expressed defenses (Van Hulten et al., 2006). Intriguingly, the fitness benefits of priming outweighed its costs under pathogen pressure, suggesting that priming may function as an ecological adaptation of the plant to respond faster to its hostile environment.

Differential signal signatures, pathway cross-talk, attacker-mediated suppression of host defense signaling, and priming for enhanced defense are major molecular mechanisms by which the defense response of the plant is shaped. Unraveling the complexity of these mechanisms and their contribution to the plant's adaptive response to the often hostile environment is a major challenge for future research in the field of molecular plant-microbe interactions.

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