Molecular Mechanisms Involved in Induced Resistance Signaling in Arabidopsis

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Abstract

Evolution has provided plants with sophisticated defensive strategies to "perceive" attack by pathogens and insects, and to translate that "perception" into an appropriate adaptive response. Plant innate immunity is based on a surprisingly complex response that is highly flexible in its capacity to recognize and respond to the invader encountered. In the past years, we explored Arabidopsis as a model to study the molecular basis of rhizobacteria-induced systemic resistance (ISR). We discovered novel components of the ISR signaling pathway and revealed that priming for augmented expression of pathogen-responsive genes plays an important role in this type of induced resistance. Currently our research is also focused on the question: how are plants capable of integrating microbial- and insect-induced signals into defense responses that are specifically directed against the attacker? The alarm signals salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are major regulators of plant defense. Their signaling pathways cross-communicate, providing the plant with a regulatory potential to fine-tune its defense reaction. We discovered that the regulatory protein NPR1 functions as a modulator in cross-talk between SA and JA, thereby helping the plant to "decide" which defensive strategy to follow, depending on the type of attacker encountered, and that this function of

NPR1 is conserved among Arabidopsis accessions all over the world, suggesting an important role for plant survival.

Rhizobacteria-Induced Systemic Resistance

SCREENING OF Ac/Ds ENHANCER TRAP LINES

Plants develop an enhanced defensive capacity against a broad spectrum of plant pathogens after colonization of the roots by selected strains of non-pathogenic, fluorescent *Pseudomonas* spp. (Pieterse et al. 2002; Van Loon et al. 1998). In *Arabidopsis thaliana*, this rhizobacteria-induced systemic resistance (ISR) functions independently of salicylic acid (SA) but requires responsiveness to jasmonic acid (JA) and ethylene (ET) (Pieterse et al. 1998). In contrast to pathogen-induced systemic acquired resistance (SAR), rhizobacteria-mediated ISR is not associated with changes in the expression of pathogenesis-related (*PR*) genes (Pieterse et al. 1996). To identify genes that are specifically expressed in response to colonization of the roots by ISR-inducing *Pseudomonas fluorescens* WCS417r bacteria, we screened a collection of Arabidopsis enhancer trap lines containing a transposable element of the *Ac/Ds* system and the *GUS* reporter gene (Léon-Kloosterziel et al. 2005). We identified an enhancer trap line that specifically showed GUS activity in the root vascular bundle upon colonization of the roots by WCS417r. The ET precursor 1-aminocyclopropane-1-carboxylate (ACC) mimicked the rhizobacteria-induced *GUS* expression pattern in the root vascular bundle, indicating that the *Ds* element was inserted in the vicinity of an ET-responsive gene. Analysis of the genes in the close vicinity of the *Ds* element revealed *AtTLP1* as the gene responsible for the in *cis* activation of the *GUS* reporter gene in the root vascular bundle. *AtTLP1* encodes a thaumatin-like protein that belongs to the PR-5 family of pathogenesis-related proteins, some of which possess antimicrobial properties.

THE TRANSCRIPTOME OF ISR

To identify ISR-related genes, we surveyed the transcriptional response of over 8,000 Arabidopsis genes during ISR (Verhagen et al. 2004). Locally in the roots, ISR-inducing WCS417r bacteria elicited a substantial change in the expression of 97 genes. However, systemically in the leaves, none of the ~8,000 genes tested showed a consistent change in expression in response to colonization of the roots by WCS417r, indicating that the onset of ISR in the leaves is not associated with detectable changes in gene expression. After challenge inoculation of WCS417r-induced plants with pathogenic

Pseudomonas syringae pv. *tomato* DC3000 (*Pst* DC3000), 81 genes showed an augmented expression pattern in ISR-expressing leaves, suggesting that these genes were primed to respond faster and/or more strongly upon pathogen attack. The majority of the primed genes was predicted to be regulated by JA and/or ET signaling (Verhagen et al. 2004). Priming of pathogen-induced genes has also been implicated in other types of induced resistance (Conrath et al. 2002; Kohler et al. 2002; Ton et al. 2005), and is thought to allow the plant to react more effectively to the invader encountered. Hence, the priming phenomenon might explain the broad-spectrum action of rhizobacteria-mediated ISR.

MYB72 IS REQUIRED IN THE ROOTS FOR SYSTEMIC EXPRESSION OF ISR

GeneChip analysis of roots uncovered 97 WCS417r-responsive, root-specific genes. To identify their role in ISR signaling, we analyzed knockout mutants of a subset of these genes. Analysis of selected T-DNA insertion lines revealed that AtMYB72, a R2R3-MYB-like transcription factor protein, is essential for activation of ISR. *AtMYB72* is specifically activated in the roots upon colonization by WCS417r. *AtMYB72* expression could also be induced by exogenous application of ACC. Disruption of the *AtMYB72* gene by T-DNA insertion rendered the knockout mutant plants incapable of mounting WCS417r-mediated ISR against the challenging pathogens *Pst* DC3000 and *Hyaloperonospora parasitica*. WCS417r-induced expression of *AtMYB72* was abolished in the ET-insensitive, ISR-nonresponsive mutant *ein2-1*, but not in the ISR-responsive, SA-defective transformant NahG. Moreover, AtMYB72 was found to physically interact *in vitro* with the ET-regulatory protein EIL3. These results indicate that induction of ISR by root-colonizing WCS417r-bacteria is controlled by the MYB72 transcription factor in the roots, and that AtMYB72 is an intrinsic part of local, ET-dependent signaling events that eventually lead to systemic expression of ISR in the leaves.

MYC2 IS INVOLVED IN PRIMING FOR JA-RESPONSIVE GENE EXPRESSION DURING ISR

GeneChip analysis of leaves showed that Arabidopsis plants expressing WCS417r-mediated ISR are primed to express specific sets of genes faster or at a higher level upon attack by virulent *Pst* DC3000. Notably, ISR constitutes a reinforcement of extant JA/ET-dependent basal defense responses against *Pst* DC3000 (Ton et al. 2002). In agreement with this, it was demonstrated that ISR-expressing tissues are primed to respond faster and stronger to JA (Pozo et al. 2005). Analysis of the promoter sequences of

the JA-responsive, ISR-primed genes revealed that they are significantly enriched in binding sites for the transcription factor AtMYC2, a key regulator of the JA signaling pathway (Lorenzo et al. 2004). Phenotypic and molecular analysis of plants overexpressing or lacking a functional AtMYC2 protein confirmed the role of this transcription factor in priming during ISR.

ISR IS EFFECTIVE AGAINST A GENERALIST HERBIVORE, BUT NOT AGAINST A SPECIALIST

To investigate whether ISR in Arabidopsis is effective against herbivorous insects as well, we examined the effect of WCS417r-mediated ISR against the generalist herbivore *Spodoptera exigua* and the specialist herbivore *Pieris rapae*. Performance of *S. exigua* larvae was significantly reduced on ISR-expressing plants, resulting in a slower gain in fresh weight. The performance of *P. rapae* was not affected on ISR-expressing plants. To investigate the mechanism of the resistance observed against the generalist herbivore, we analysed the expression of a set of well-characterized, defense-related genes in control and ISR plants, before and after infestation with *S. exigua* or *P. rapae*. Northern blot analysis showed that *S. exigua* feeding induced the JA-responsive gene *PDF1.2* to a much higher level in ISR-expressing plants than in non-induced control plants. These suggest that priming for JA-responsive gene expression, which is associated with ISR, contributes not only to pathogen resistance, but also to resistance against herbivorous insects. This priming effect was not observed after feeding by *P. rapae*, which is in agreement with the ineffectiveness of ISR against this herbivore, and suggests that this specialist somehow circumvents this defense response.

Cross-Talk Between Signaling Pathways to Fine-Tune Defense

SIGNAL SIGNATURE AND TRANSCRIPTOME CHANGES DURING PATHOGEN AND INSECT ATTACK

To understand how plants integrate pathogen- and insect-induced signals into specific defense responses, we monitored the dynamics of SA, JA, and ET signaling in *Arabidopsis* after attack by a set of microbial pathogens and herbivorous insects with different modes of attack (De Vos et al. 2005). *Arabidopsis* plants were exposed to a pathogenic leaf bacterium (*Pst* DC3000), a pathogenic leaf fungus (*Alternaria brassicicola*), tissue-chewing caterpillars (*P. rapae*), cell-content-feeding thrips (*Frankliniella occidentalis*), or phloem-feeding aphids (*Myzus persicae*). Monitoring the

signal signature in each plant-attacker combination showed that the kinetics of SA, JA, and ET production varies greatly in both quantity and timing. Analysis of global gene expression profiles demonstrated that the signal signature characteristic of each *Arabidopsis*-attacker combination is orchestrated into a surprisingly complex set of transcriptional alterations. Comparison of the transcript profiles revealed that consistent changes induced by pathogens and insects with very different modes of attack can show considerable overlap. Of all consistent changes induced by *A. brassicicola*, *P. rapae*, and *F. occidentalis*, more than 50% were also induced consistently by *Pst* DC3000. Notably, although these four attackers all stimulated JA biosynthesis, the majority of the changes in JA-responsive gene expression were attacker-specific. All together these results show that SA, JA, and ET play a 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.

DIFFERENTIAL EFFECTIVENESS OF HERBIVORE-INDUCED RESISTANCE AGAINST MICROBIAL PATHOGENS

Caterpillars of the herbivore *P. rapae* stimulate the production of JA and ET in Arabidopsis (De Vos et al. 2005), and trigger a defense response that affects insect performance on systemic tissues. To investigate the spectrum of effectiveness of this herbivore-induced defense response, the level of protection against different microbial pathogens was examined. Caterpillar feeding significantly reduced disease caused by *Pst* DC3000 and *Xanthomonas campestris*. Although the necrotrophic fungus *A. brassicicola* is sensitive to JA-dependent defenses, herbivore-induced resistance was not effective against this pathogen. *PDF1.2*, a JA-responsive marker gene for *A. brassisicola* resistance, was suppressed by the regurgitant of *P. rapae*, suggesting that *P. rapae* antagonizes this JA-dependent defense response. Resistance against turnip crinkle virus (TCV) requires SA, but not JA and ET. Nevertheless, herbivore feeding strongly affected TCV multiplication and TCV lesion formation, also in systemic tissues. Wounding alone was not effective, but application of regurgitant onto the wounds induced a similar level of protection. Analysis of SA-induced *PR-1* expression revealed that *P. rapae* grazing primes Arabidopsis leaves for augmented expression of SA-dependent defenses. Herbivore-induced ET production was shown to play positive a role in this process. These results demonstrate that insect feeding triggers a surprisingly complex defense response, and provide evidence that both synergistic and antagonistic effects of pathway cross-talk shape the outcome of the resistance reaction.

NPR1-DEPENDENT CROSS-TALK BETWEEN SA AND JA IS CONSERVED AMONG ARABIDOPSIS ACCESSIONS

SA has an antagonistic effect on JA signaling, a process called pathway cross-talk that is believed to allow the plant to fine-tune its defense response, depending on the attacker encountered. Analysis of the mutant *npr1,* which is impaired in SA signal transduction, revealed that SA-mediated down-regulation of JA-responsive gene expression requires the regulatory protein NPR1 (Pieterse and Van Loon 2004; Spoel et al. 2003). Furthermore, we have shown that nuclear localization of NPR1, which is essential for SAR, is not required for the suppression of JA signaling, indicating that cross-talk between SA and JA signaling pathways is modulated through a novel function of NPR1 in the cytosol. We have further investigated the cross-talk phenomenon by exploring naturally occurring variation in pathway cross-talk using Arabidopsis accessions that were collected all over the world from very different habitats. Our results demonstrate that the antagonism between SA and JA signaling is conserved among Arabidopsis accessions, suggesting an important role for plant survival.

Acknowledgments

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