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Article Addendum

Regulation of defense gene expression by Arabidopsis *SRFR1*

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Reduced growth and viability is a common phenotype of plants with constitutively activated pathogen defenses. One branch of the plant innate immunity system, effector-triggered immunity, is especially potent and requires tight control to enable normal plant development. While some facets of this control that directly regulate resistance protein abundance or activity have been documented, general control of effector-triggered signaling sensitivity is poorly understood. We recently identified *SUPPRESSOR OF rps4-RLD 1* (*SRFR1*), a novel negative regulator of *avrRps4*-triggered immunity. Mutations in *SRFR1* were previously shown not to induce constitutive high expression of the defense gene *PR1*, and to be fully susceptible to the virulent *Pseudomonas syringae* pv. tomato strain DC3000. *SRFR1* encodes a tetratricopeptide repeat-containing protein with weak similarity to transcriptional repressors in other organisms. By transient expression in *Nicotiana benthamiana*, SRFR1 was localized to the nucleus. Here we investigate more carefully whether expression of defense genes is misregulated in *srfr1* mutant plants. Consistent with the hypothesized function of SRFR1 as a negative transcriptional regulator, we find that mRNA levels of several defense genes are upregulated in *srfr1* mutants.

Effector-Triggered Immunity

Effector-triggered immunity (ETI), which relies on the direct or indirect detection of pathogen effector proteins by host resistance (R) proteins, can lead to localized programmed cell death called the hypersensitive response (HR).[1-4] Even when ETI is not accompanied by HR, in Arabidopsis the detrimental effects of the resistance response can be evident as chlorosis.[5] As a result, stunted growth and poor seed-set are common phenotypes associated with constitutive ETI responses. Consequently, the plant must fine-tune the response to pathogens by exerting tight positive and negative control.[6]

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SRFR1 is a Novel Regulator of ETI

By performing a genetic suppressor screen, we previously identified mutants with specifically enhanced responses to the *P. syringae* effector protein AvrRps4. The Arabidopsis accession RLD is a natural mutant in the cognate R protein RPS4 and is fully susceptible to DC3000 expressing *avrRps4*.[7,8] Mutations in RLD in a gene we called *SUPPRESSOR OF rps4-RLD 1* (*SRFR1*) enhanced resistance to DC3000 (*avrRps4*), but not to virulent DC3000. The mutant *srfr1* alleles were recessive, suggesting that genetically *SRFR1* functions as a negative regulator of AvrRps4-triggered immunity.[9]

SRFR1 encodes a novel tetratricopeptide repeat-containing protein that is conserved between plants and animals. To date none of the proteins in other organisms have an assigned function. SRFR1 orthologs appear to be absent in *Saccharomyces cerevisiae*, *Caenorhabditis elegans* and *Drosophila melanogaster*. While proteins that share amino acid sequence similarity over the whole length of SRFR1 are absent in these organisms, the SRFR1 TPR domain shares some sequence similarity with the TPR-containing transcriptional repressors ScSSN6 and CeOGT1. We therefore proposed that AtSRFR1 functions as a transcriptional repressor of plant defense genes that fine-tunes the Arabidopsis defense response.[10]

Altered Expression of Defense Genes in *srfr1* Mutants

Initial characterization of *srfr1* mutants by RNA gel blots showed that the defense gene *PR1* is not constitutively upregulated, consistent with the absence of elevated resistance to virulent DC3000.[9] After cloning of *SRFR1*, we quantified the expression of *RPS4* in the mutants and determined that *RPS4* mRNA levels, which are induced approximately 10-fold by *avrRps4* in resistant wild-type plants,[11] are approximately two-fold higher in uninduced *srfr1* mutants compared to uninduced wild-type plants.[10] This raised the possibility that other defense-related genes are also slightly upregulated in *srfr1* mutants, albeit not highly enough to trigger constitutive activation of plant defenses. This would be consistent with our model that *srfr1* mutants are closer to a threshold for defense activation.

Several defense-related genes were indeed upregulated in uninduced *srfr1-1* and *srfr1-2* leaf tissue, including *PR1* (Fig. 1A). *PR1* expression has been reported to be induced over 1,000-fold in resistant plants upon pathogen inoculation,[12] and the level of *PR1* induction in uninduced *srfr1* mutants was still considerably lower than in SA-induced tissue from a separate batch of wild-type or mutant plants (Fig. 1B). This perhaps explains why lower levels of

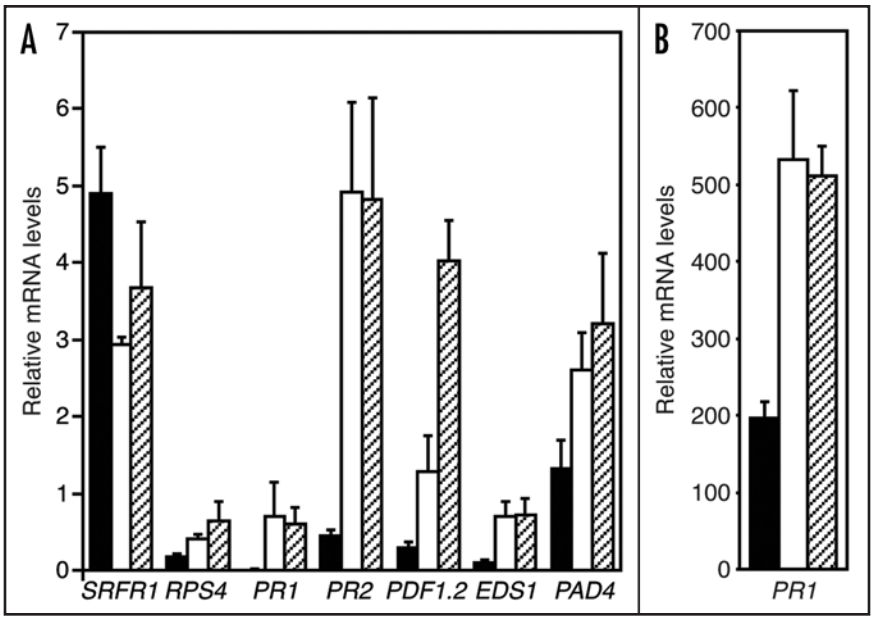

Figure 1. Defense gene mRNA levels are higher in *srfr1* mutants than in the wild-type. Total RNA was isolated from three biological replicates. mRNA levels were quantified by real-time quantitative reverse transcription PCR and were normalized using *SAND* gene (At2g28390) mRNA levels as an internal standard.[10] Error bars denote standard error. (A) Defense gene mRNA levels in uninduced tissue of RLD wild-type (closed bars), *srfr1-1* (open bars) and *srfr1-2* (hatched bars). (B) *PR1* mRNA levels 24 h after induction by spraying leaf tissue of RLD (closed bars), *srfr1-1* (open bars) and *srfr1-2* (hatched bars) with 1.5 mM SA. Note difference in scale. Primers used were: 5'-AAC TCT ATG CAG CAT TTG ATC CAC T-3' and 5'-TGA TTG CAT ATC TTT ATC GCC ATC-3' for *SAND*; 5'-CTG GAT ATG CCT CAC TAG AAG-3' and 5'-CAC TGG GTC ACA AGG CTC TG-3' for *SRFR1* (At4g37460); 5'-CCT AAC ATT ATG GGC ATC ATC A-3' and 5'-CCG CCT TCA CAA TTT CAT TGA-3' for *RPS4* (At5g45250); 5'-GCA ATG GAG TTT GTG GTC AC-3' and 5'-GTT CAC ATA ATT CCC ACG AGG-3' for *PR1* (At2g14610); 5'-ATC TCC CTT GCT CGT GAA TC-3' and 5'-GGA TCG TTA TCA ACA GTG GAC-3' for *PR2* (At3g57260); 5'-AAG TTG TGC GAG AAG CCA AG-3' and 5'-CCA TGT TTG GCT CCT TCA AG-3' for *PDF1.2* (At5g44420); 5'-GAC GGG GAA GTA GAT GAG AAG-3' and 5'-TCA TCC ATC ATA CGC TCA CG-3' for *EDS1* (At3g48080); and 5'-GAG GAG ATC TTT GTT ACG GG-3' and 5'-TCG CCT CCC ACA CAC TAT AA-3' for *PAD4* (At3g52430).

PR1 induction in *srfr1* mutants was previously not detected by RNA gel blot analysis.[10]

In summary, we have found additional support for our model that SRFR1 function determines a set-point in the plant innate immune response system by negatively regulating defense gene expression levels. In this model, weak recognition of AvrRps4 in the natural *RPS4* mutant RLD is not sufficient to trigger resistance because of the suppressive function of SRFR1. In *srfr1-1* and *srfr1-2* plants on the other hand, weak recognition of AvrRps4 is sufficient to exceed a threshold for resistance activation. Whether SRFR1 directly downregulates defense gene expression, or whether this regulation is indirect, awaits further study.

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