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Article Addendum

Histone modifications do not play a major role in salicylate-mediated suppression of jasmonate-induced *PDF1.2* gene expression

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Cross-talk between salicylic acid (SA) and jasmonic acid (JA) defense signaling pathways allows a plant to finely tune its response to the attacker encountered. In Arabidopsis, pharmacological experiments revealed that SA exerts a strong antagonistic effect on JA-responsive genes, such as *PDF1.2,* indicating that the SA pathway can be prioritized over the JA pathway. We investigated the putative role of histone modifications in the regulation of SA-mediated suppression of *PDF1.2* transcription. Chromatin immunoprecipitation analysis using an antibody directed against acetylated histone H3 revealed that SA does not affect the association of this histone modification at the *PDF1.2* promoter, suggesting that chromatin remodeling does not play a major role in SA/JA cross-talk.

Plants can activate specific defense responses in order to resist attack by deleterious organisms. Apart from pre-existing chemical or structural barriers, inducible defenses are triggered, which often act systemically throughout the plant and confer broad-spectrum resistance. The plant hormones salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) play important roles in the regulation of these induced defenses.[1-7] The corresponding signal transduction pathways cross-communicate, providing the plant with a highly flexible defense signaling network. Cross-talk between signaling pathways is thought to optimize the defense reaction to a particular attacker by enhancing the appropriate response, while suppressing suboptimal reactions. Indeed, trade-offs between defense signaling pathways have been demonstrated in several plant species.[8-10]

Elucidation of the molecular mechanism underlying this pathway cross-talk will provide insight into how a plant copes with multiple stress inputs and relays these to an appropriate defense reaction.[11,12] Several key regulatory proteins involved in SA-mediated suppression of JA-responsive genes have been identified in *Arabidopsis thaliana*.[13] Previously, we demonstrated that the defense regulatory protein NPR1 is required for this SA/JA cross-talk.[14] In mutant *npr1-1* plants, the SA-mediated suppression of JA-responsive gene expression was completely abolished. 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 node of convergence between both pathways.[15,16] Recently, overexpression of the SA-regulated glutaredoxin GRX480 was found to antagonize JA-responsive *PDF1.2* transcription.[17] Furthermore, a role for redox modulation was suggested in the SA/JA antagonism.[12]

To identify novel key players in SA/JA cross-talk we assessed whether chromatin remodeling plays a role in the SA-mediated downregulation of JA-responsive genes. DNA is packaged with histone proteins into chromatin, which physically restricts the accessibility of the genome to regulatory proteins, such as transcription factors. The chromatin configuration can be altered to allow or prevent access of the transcription machinery by covalent modifications of the exposed N-terminal histone tails in the nucleosome. Histone acetylation is mediated by the activity of histone acetyltransferases (HATs) and is often associated with increased gene activity. Histone deacetylation, mediated by histone deacetylases (HDACs), and methylation are generally correlated with transcriptional repression.[18,19] Thus, gene expression can be regulated at the level of histone modifications. Previously, a HDAC was found to interact with the JA regulatory protein COI1.[20] The expression of this gene, as well as another HDAC, *HDA19*, was shown to be upregulated by JA and the ET precursor 1-aminocyclopropane-1-carboxylic acid (ACC).[21] Overexpression of *HDA19* enhanced expression of several JA- and ET-responsive genes and conferred increased resistance to the necrotrophic pathogen *Alternaria brassicicola*.[21] Chromatin immunoprecipitation analysis demonstrated that induction of SA-responsive *PR-1* gene expression is associated with an increase in histone acetylation at the *PR-1* promoter in Arabidopsis and tobacco,[22,23] suggesting involvement of histone modifications in the activation of SA-responsive genes too. Since both SA-responsive and JA-responsive defense signaling pathways involve chromatin remodeling,[21-23] we investigated the potential

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influence of histone acetylation on the SA-mediated suppression of JA-responsive gene expression.

Five-week-old *A. thaliana* wild-type Col-0 and mutant *npr1-1* plants were treated with an aqueous solution of 1 mM SA, 0.1 mM MeJA, or a combination of both chemicals as described previously.[12] Leaf tissue was harvested 24 h after induction, and the expression of SA-responsive *PR-1* (At2g14610) and MeJA-responsive *PDF1.2* (At5g44420) marker genes was assessed. As expected, SA induced *PR-1* expression and suppressed MeJA-induced expression of *PDF1.2* in Col-0 plants (Fig. 1A). Both induction of *PR-1* and suppression of *PDF1.2* by SA was blocked in mutant *npr1-1,* confirming that the plant material displayed NPR1-dependent SA/JA cross-talk (Fig. 1A).[14] This plant material was subjected to chromatin immunoprecipitation (ChIP) analysis,[7] using an antibody directed against acetylated histone H3 (AcH3; 06-599; Upstate, Lake Placid, USA), which is a marker for a more transcriptionally permissive state.[19,24] RT-Q-PCR analysis was performed on 1.5 μL immunoprecipitated and non-immunoprecipitated input DNA with primers designed to amplify fragments of the *PR-1* promoter (*PR-1* Fw; 5' TCG GTC CCT AGA GTT TTT CAA 3' and *PR-1* Rv; 5' CCG CCA CAT CTA TGA CGT AAG 3') and the *PDF1.2* promoter (*PDF1.2* Fw; 5' TTC AGT AAT AGG TGT GTC CCA GG 3' and *PDF1.2* Rv; 5' GCG GCT GGT TAA TCT GAA TGG 3'). As a control, primer sets were included that amplify promoter fragments of two constitutively expressed marker genes, *GAPDH* (*GAPDH* Fw; 5' GCA AAG CTC ATT GGC TGT CA 3' and *GAPDH* Rv; 5' GGA AAC TAA TGG CGC TTG GA 3') and *UBQ10* (*UBQ10* Fw; 5' TTG CCA ATT TTC AGC TCC AC 3' and *UBQ10* Rv; 5' TGA CTC GTC GAC AAC CAC AA 3').[25] The amount of immunoprecipitated DNA was calculated relative to the input DNA using the $2^{-\Delta\Delta C_T}$ method.[26] The C_T values of input and ChIP DNA were averaged before performing the ΔC_T calculation, and the variance estimated from the replicate C_T values was carried through to the final calculation of relative quantities using standard propagation of error methods. The error was estimated by calculating the $2^{-\Delta\Delta C_T}$ term using ΔC_T plus the standard deviation and ΔC_T minus the standard deviation.[26] Next, the amount of immunoprecipitated DNA was corrected for dilution factors, and control-treated samples of Col-0 and *npr1-1* were set at 1.

Figure 1B shows that SA treatment resulted in an increase of AcH3 at the *PR-1* promoter, confirming previous findings with the SA analog benzothiadiazole S-methyl ester (BTH) as the inducing agent.[22] This increase was absent in *npr1-1*, suggesting that SA-mediated acetylation of histone H3 at the *PR-1* promoter is NPR1-dependent. However, the combined treatment with SA and MeJA did not result in AcH3 enrichment at the *PR-1* promoter, while the *PR-1* gene was normally expressed (Fig. 1A and B). Therefore, the chromatin structure around the *PR-1* promoter appears to be sufficiently relaxed to allow access to the transcriptional machinery. This notion is supported by data on the epigenetic regulation of the WRKY70 transcription factor.[27] It was suggested that the chromatin structure of WRKY70 target genes, such as *PR-1*, is sufficiently open to allow a fast response to stimuli. Consequently, by epigenetic regulation of a single transcription factor, the expression of a large number of genes can be affected, thus significantly increasing the regulatory potential of chromatin remodeling.[27]

In contrast to the *PR-1* promoter, AcH3 association at the *PDF1.2* promoter did not surmount the fluctuation observed at the promoters of the two constitutively expressed genes, *GAPDH* and *UBQ10* (Fig. 1B). Hence, the negative effect of SA on MeJA-induced expression of *PDF1.2* did not correlate with changes in AcH3 histone modification. These data suggest that chromatin remodeling does not play a major role in the regulation of SA/JA cross-talk. Conclusive evidence should be provided by additional experiments and include analysis with antibodies directed against multiple histone modifications.

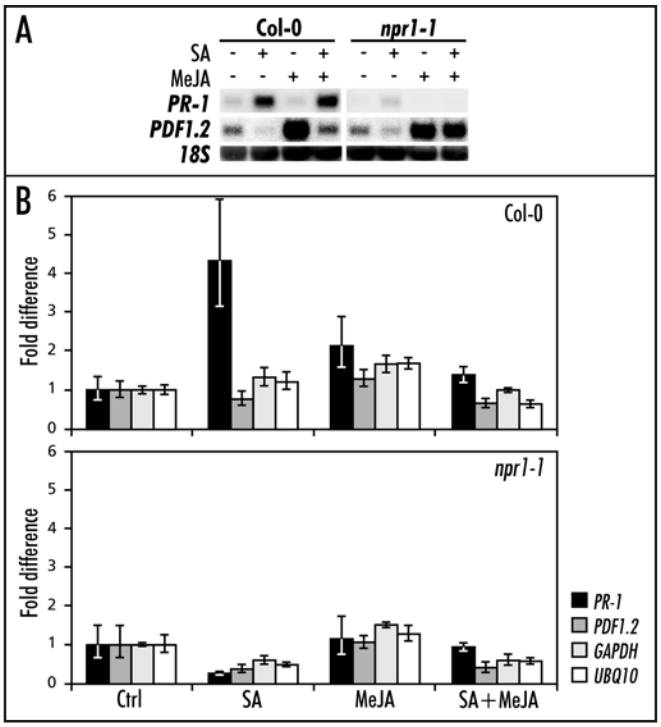

Figure 1. Chromatin immunoprecipitation (ChIP) analysis of histone H3 acetylation at the *PR-1* and *PDF1.2* promoters in Col-0 and *npr1-1*. (A) Northern blot analysis of *PDF1.2* and *PR-1* expression in Col-0 and *npr1-1* leaves treated with 1 mM SA, 0.1 mM MeJA, or a combination of both chemicals. Leaf tissue was harvested 24 h after chemical treatment. Equal loading of RNA samples was checked using a probe for *18S* rRNA. (B) ChIP analysis of Col-0 and *npr1-1* treated with 1 mM SA, 0.1 mM MeJA, or a combination of both chemicals. Chromatin samples were subjected to immunoprecipitation using an AcH3 antibody. The immunoprecipitated DNA was analyzed for the enrichment of *PR-1*, *PDF1.2*, *UBQ10* and *GAPDH* promoter sequences by Q-RT-PCR. The fold increase of immunoprecipitated DNA was calculated relative to the input DNA.

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