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The epigenetic machinery controlling transgenerational systemic acquired resistance

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Progeny from diseased Arabidopsis shows enhanced resistance, which is associated with priming of defense genes.[1] This transgenerational systemic acquired resistance (SAR) is effective against biotrophic pathogens, such as the downy mildew pathogen *Hyaloperonospora arabidopsidis*. In this study, we have examined mutants in RNA-directed DNA methylation (RdDM) for transgenerational SAR. Our analysis suggests that transgenerational SAR is regulated by the RdDM pathway and transmitted by hypomethylation at CpNpG sites.

Priming of defense is an adjustment of the plant's immune system, which results in a faster and stronger activation of inducible defense mechanisms after exposure to environmental stress.[2-4] A well-characterized form of defense priming takes place during expression of systemic acquired resistance (SAR). This resistance response is effective against biotrophic pathogens, requires regulation by the defense regulatory gene *NON EXPRESSOR OF PR GENES (NPR1*), and is associated with priming of salicylic acid (SA)-dependent genes.[5,6] Recent evidence suggests that priming of SA-inducible genes involves epigenetic regulatory mechanisms, such as post-translational modifications of histone proteins and the RNA Polymerase V.[7,8]

We recently demonstrated that progeny from *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000-infected Arabidopsis (P_1 progeny) are primed for SA-dependent defense compared with progeny from control-treated healthy plants (C_1 progeny). We named this phenomenon "transgenerational SAR", since the resistance in P_1 progeny is effective against (hemi)-biotrophic pathogens, it requires *NPR1,* and it is associated with priming of SA-inducible defense genes.[1] In the same journal issue, two complementary publications demonstrated transgenerational defense priming upon exposure to herbivory and the chemical priming agent β-aminobutyric acid.[9,10] Transgenerational SAR in P_1 progeny from diseased plants was associated with increased levels of acetylated histone 3 at lysine 9 (H3K9) at SA-inducible gene promoters,[1] a chromatin mark that is associated with a permissive state of transcription.[11] Moreover, the *drm1drm2cmt3* (*ddc*) triple mutant, which is affected in non-CpG DNA methylation,[12] mimicked the transgenerational SAR phenotype.[1] Since infection by *Pst*DC3000 induces DNA hypomethylation in Arabidopsis,[13] we hypothesized that transgenerational SAR is transmitted through DNA hypomethylation at non-CpG sites.

In this study, we have tested transgenerational SAR phenotypes of various Arabidopsis mutants in the RNA-directed DNA methylation (RdDM) pathway (**Figs. 1 and 2**). Parental plants were repeatedly inoculated with either mock solution, or a suspension containing *Pst*DC3000 bacteria, and allowed to set seeds. At least three independent C_1 and P_1 progenies of each line were tested for resistance against the downy mildew pathogen *Hyaloperonospora arabidopsidis*, as described before in reference 1. The results of these bioassays are summarized in **Figure 2**.

P_1 progeny from the RdDM pathway mutants *ago4-3*,[14] *clsy1-1*,[15] *nrpd2a-1*,[16] *drd1-6* [17] and *dcl3-1*,[18] failed to express increased resistance in comparison to their C_1 progenies. Furthermore, C_1 progeny of the *ago4-3* mutant showed constitutively enhanced resistance in comparison to C_1 progeny of wild-type plants (χ^2 = 14.2; p = 0.002). Since mutations in *AGO4* cause reduced levels of non-CpG DNA methylation,[19] the phenotype of the *ago4-3* mutant supports our hypothesis that hypomethylation at non-CpG sites transmits SAR. Surprisingly, however, C_1 progeny from the other RdDM mutants displayed similar levels of susceptibility as C_1 progeny from wild-type plants. Considering their inability to express transgenerational SAR, these phenotypes suggest positive regulation by the corresponding RdDM components. In a second experiment, we tested additional Arabidopsis mutants impaired in non-CpG DNA methyltransferase activity. As observed for the *ago4-3* mutant and *ddc* triple mutant,[1] C_1 and P_1 progenies from the *drm1drm2kyp (ddk)* triple mutant[12] showed no difference in resistance to *H. arabidopsidis*, while the C_1 progeny of this triple mutant expressed elevated levels of basal resistance in comparison to C_1 progeny from wild-type plants (χ^2 = 17.31; p = 0.001). By contrast, the *drm1drm2 (dd)* double mutant, which is specifically impaired in asymmetric CpHpH DNA methylation,[12] displayed a wild-type phenotype and was unaffected in transgenerational SAR and basal resistance.

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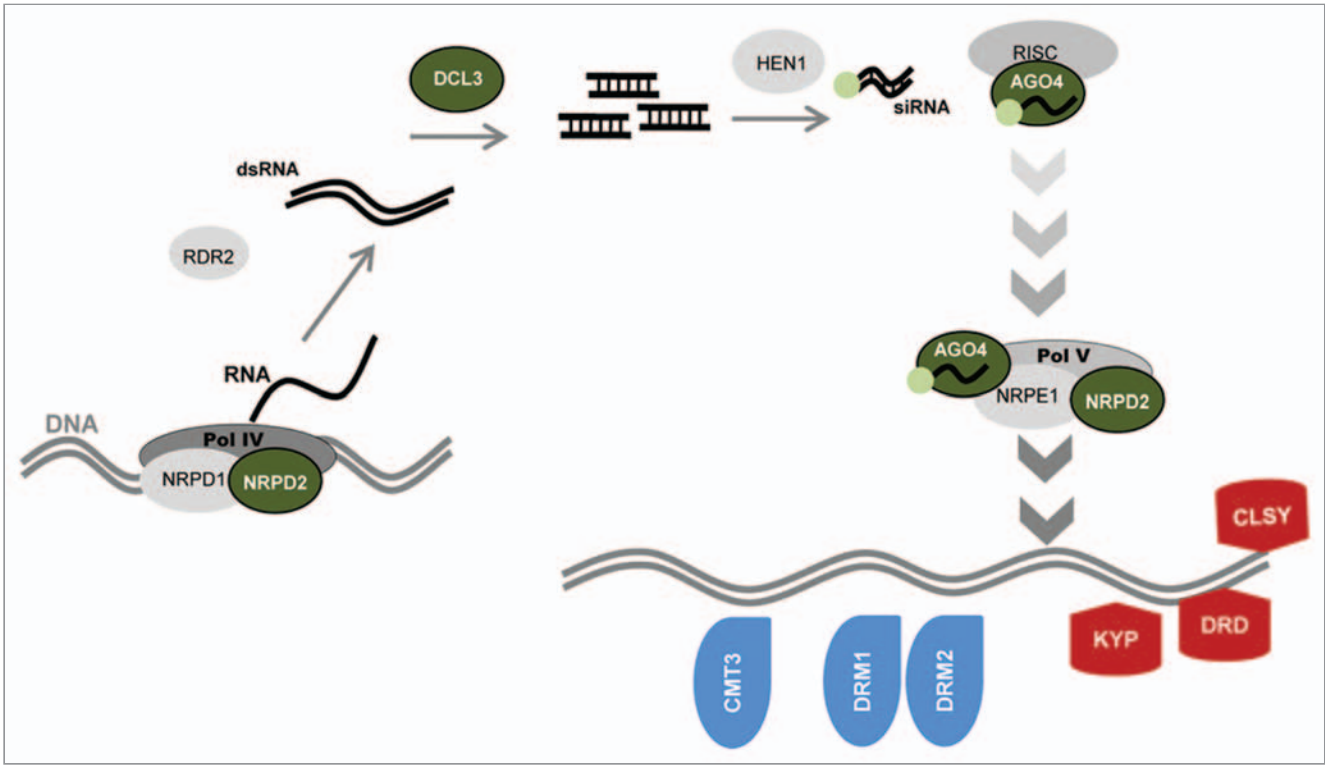

Figure 1. Model of the RNA-directed DNA methylation (RdDM) pathway, adapted from references 25 and 26. Multi-subunit DNA-dependent RNA Polymerase IV (Pol IV) generates single stranded RNAs, which are used as a template by RNA-dependent RNA polymerase 2 (RDR2) to generate double-stranded RNAs (dsRNAs). *DICER-LIKE 3* (*DCL3*) processes dsRNAs into 24-nucleotide siRNAs that become methylated by the RNA-methyltransferase *HEN1* and subsequently recruited by the *AGO4-RISC* complex to targeted genomic sites via interaction with the DNA-dependent RNA Polymerase V (Pol V). Pol V is required for DNA methyltransferase activity by *DRM1*, *DRM2* and *CMT3* and chromatin remodeling enzymes *DRD*, *CLSY* and *KYP*. The RdDM pathway targets cytosine (C) methylation at non-CpG sites. Signaling components affected in the mutants tested for transgenerational SAR are shown in color; green, enzymes in the siRNA machinery; red, chromatin remodeling enzymes; blue, DNA methyltransferase enzymes.

Finally, the *kyp-6*,[12] single mutant, which is specifically affected in CpHpG methylation,[20] resembled the defense phenotype of *clsy1-1*, *nrpd2a-1*, *drd1-6* and *dcl3-1*: there was no significant difference in resistance between C_1 and P_1 progenies, and C_1 progeny of this mutant displayed similar levels of susceptibility as C_1 progeny from wild-type plants.

It is clear from the data presented in this communication that transgenerational SAR in Arabidopsis involves regulation by the RdDM pathway. The exact role of each individual RdDM component remains difficult to decipher on the basis of the presented disease phenotypes. Rasmann et al. (2012) used two RdDM pathway Arabidopsis mutants to assess the contribution of siRNAs in transgenerational priming of jasmonic-acid (JA)-dependent defense against herbivory:[9] the *nrpd2a nrpd2b* double mutant[21] and the *dcl2dcl3dcl4* triple mutant.[22] Progenies from healthy, herbivore-exposed and jasmonic acid-treated plants of these mutants failed to show differences in resistance against the specialist herbivore *Pieris rapae*, suggesting a critical role for the RdDM pathway in transgenerational priming of JA-dependent defenses. Hence, the RdDM pathway controls trans-generational priming of both JA and SA dependent defenses. We therefore propose that the RdDM pathway provides the machinery for different transgenerational defense responses, while the specificity of the response is determined by the 24-nucleotide sequence of the siRNAs.

Our findings that the *dd* double mutant expressed a wild-type phenotype, whereas C_1 and P_1 progenies of the *ddk* and *ddc* triple mutant display similar levels of constitutively enhanced resistance (**Fig. 2**),[1] points to a critical role for *KYP*- and *CMT3*-dependent DNA methylation. *KYP* directs *CMT3* activity through methylation of the lysine 9 residue of histone H3.[23] Its involvement in transgenerational SAR supports our previous observation that transgenerational SAR is marked by increased acetylation of H3K9 at SA-inducible gene promoters.[1] Since *KYP* and *CMT3* predominantly mediate cytosine methylation at symmetrical CpHpG sites,[24] we conclude that transgenerational SAR is transmitted by DNA hypomethylation at CpHpG sites.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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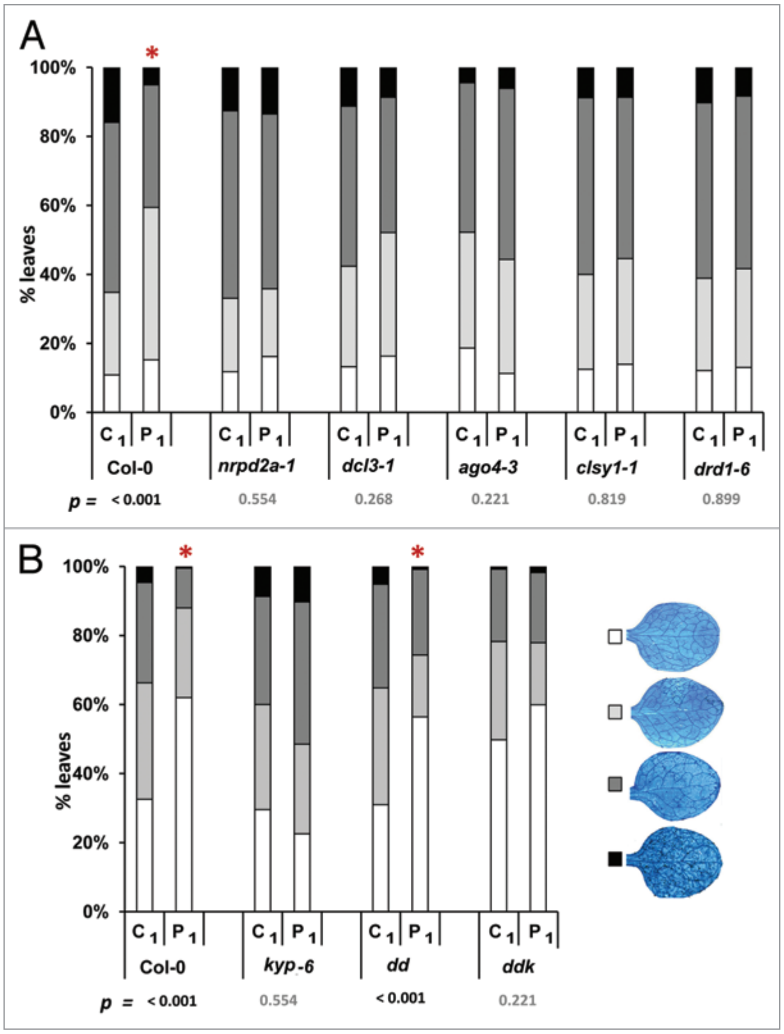

Figure 2. Transgenerational SAR against *Hyaloperonospora arabidopsidis* in mutants of Arabidopsis that are impaired in RNA-directed DNA methylation: *nrpd2a-1*, *dcl3-1*, *ago4-3*, *clsy1-1* and *drd1-6* (experiment A), and *kyp-6*, *drm1drm2 (dd)* and *drm1drm2kyp (ddk)* (experiment B). Induction of transgenerational SAR and challenge inoculation with *H. arabidopsidis* were performed as described in reference 1. At 6 d after conidiospore inoculation, stained leaves were microscopically examined and assigned to different classes, as described in reference 1. Asterisks and p values at the bottom of the graphs indicate statistical differences in class distributions between C_1 and P_1 progenies of each genotype (χ^2 Test).

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