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Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response

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Priming of defence genes for amplified response to secondary stress can be induced by application of the plant hormone salicylic acid or its synthetic analogue acibenzolar *S*-methyl. In this study, we show that treatment with acibenzolar *S*-methyl or pathogen infection of distal leaves induce chromatin modifications on defence gene promoters that are normally found on active genes, although the genes remain inactive. This is associated with an amplified gene response on challenge exposure to stress. Mutant analyses reveal a tight correlation between histone modification patterns and gene priming. The data suggest a histone memory for information storage in the plant stress response.

Keywords: chromatin; systemic acquired resistance; plant promoter control; systemic signalling

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INTRODUCTION

After localized infection by a pathogen, plants often acquire systemic immunity to further infections (Durrant & Dong, 2004). This requires the accumulation of the plant hormone salicylic acid in tissue distal from the infection site and is called systemic acquired resistance (SAR). Exogenous application of salicylic acid and some salicylic acid analogues, such as acibenzolar *S*-methyl (BTH) is sufficient to trigger resistance to biotic and abiotic stress (Ryals *et al*, 1996; Senaratna *et al*, 2000). In the SAR response, defence genes in the infected and remote tissue show the 'priming' phenomenon; they are able to respond faster and/or to a greater extent to subsequent challenge (Kohler *et al*, 2002; Conrath, 2009). The promoters of many of these genes contain at least one 'W-box' that provides binding sites for WRKY transcription factors (Maleck *et al*, 2000; Rushton *et al*, 2010). Genes encoding WRKY factors are themselves transcriptionally induced by either pathogen infection or treatment with microbe-associated molecular patterns, such as flagellin (Asai *et al*, 2002; Dong *et al*, 2003).

Mutants that are attenuated in pathogen defence are often also compromised in gene priming. For example, the *npr1* mutant of *Arabidopsis thaliana* is deficient in SAR (Durrant & Dong, 2004) and cannot be primed for enhanced gene expression (Kohler *et al*, 2002; Beckers *et al*, 2009). By contrast, defence genes are often constitutively primed for enhanced activation in mutants with permanently enhanced immunity to pathogens such as *sni1*, *cpr1* and *edr1* (Frye & Innes, 1998; Frye *et al*, 2001; Kohler *et al*, 2002; Mosher *et al*, 2006).

Chromatin structure is important for the regulation of gene expression. The basal repeat unit of chromatin is the nucleosome containing 147 base pairs of DNA wrapped around a protein core particle comprising two copies each of histones H2A, H2B, H3 and H4 (Luger *et al*, 1997). Histones are subject to many covalent modifications. Acetylation of lysines in the amino-terminal tails of histones H3 and H4 has been associated with active genes (Eberharter & Becker, 2002). This modification reduces the ionic interaction between positively charged lysine side chains and the negatively charged DNA backbone (Garcia-Ramirez *et al*, 1995). Moreover, lysine acetylation provides docking sites for transcriptional coactivator proteins containing bromodomains (Kanno *et al*, 2004). For histone methylation the situation is more complex because lysine and arginine residues can be methylated and up to three methyl groups can be added to each residue. Furthermore, specific methylation patterns are associated with both gene activation and repression. The strongest correlation between histone methylation and gene activity is found for trimethylation of Lys 4 on histone H3 (H3K4me3) on promoters and coding sequences of active genes (Ruthenburg *et al*, 2007). By contrast, the roles of dimethylation and monomethylation of the same residue in gene regulation are less defined.

Although gene priming is a widespread phenomenon and has also been described for the defence response in animals (Hayes *et al*, 1995), little is known about the mechanisms for it at the molecular level. On the basis of mutant analyses, it has been suggested recently that defence genes are poised for enhanced activation during SAR by replacement on gene promoters of

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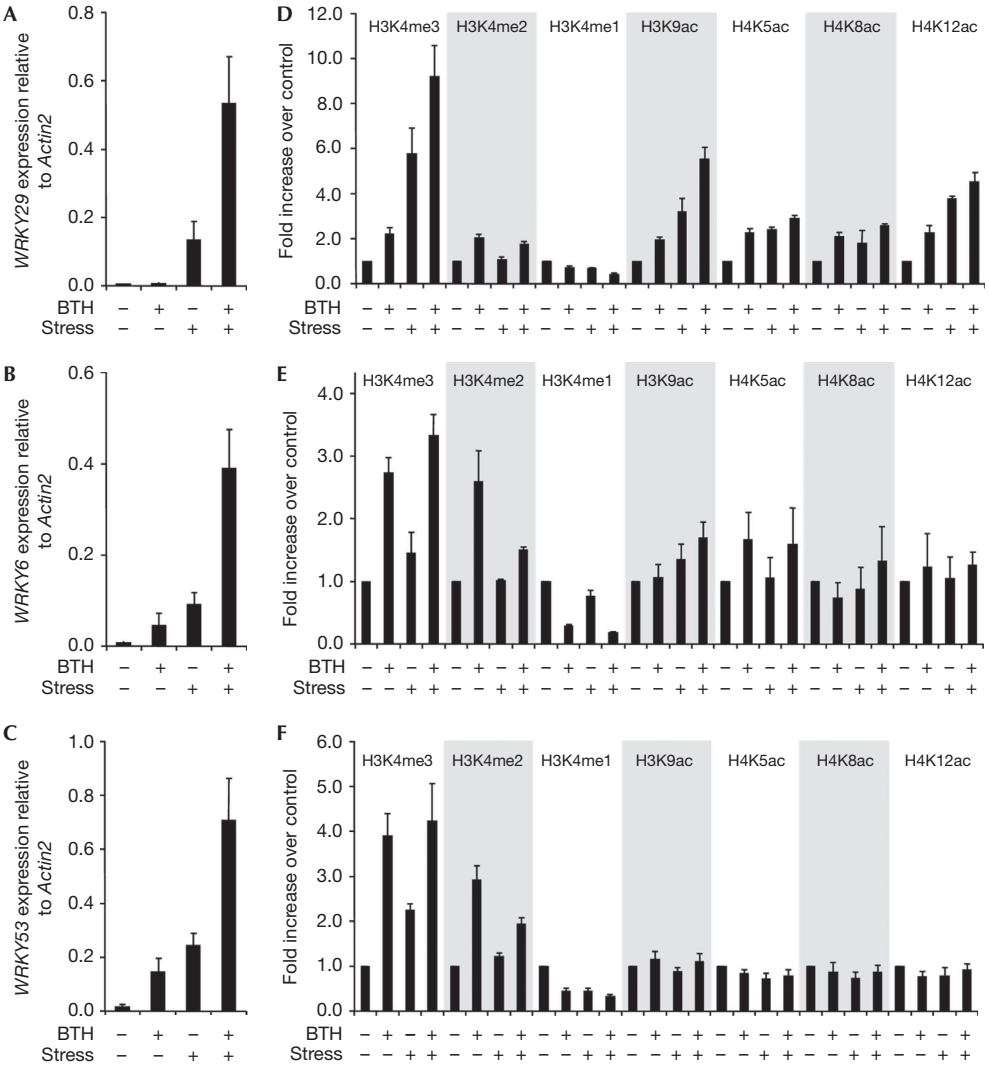

Fig 1 | Transcript abundance and histone modifications after priming and potentiated activation of three *WRKY* transcription factor genes. Plants were treated with 100 μM BTH or wettable powder (control). After 72 h, half of the plants were stressed by infiltrating water into their leaves. After 3 h, leaves were collected and RNA and chromatin were isolated. (**A–C**) Transcript abundance as determined by RT–qPCR. Data are standardized for abundance of the *Actin2* transcript. (**D–F**) H3K4 methylation (me) and histone acetylation (ac) on the gene promoters. Data are standardized for histone modification levels in the absence of inducer and stress treatment. Each data point is based on four independent replicates. Error bars indicate s.e.m. values. BTH, acibenzolar *S*-methyl; RT–qPCR, reverse transcriptase–quantitative PCR.

histone H2A with its variant H2A.Z (March-Díaz *et al*, 2008; van den Burg & Takken, 2009). In this study, we show that histone modifications—such as H3 and H4 acetylation—and H3K4 methylation are systemically set during a priming event. These modifications might create a memory of the primary infection that is associated with an amplified reaction to a second stress stimulus.

RESULTS AND DISCUSSION

Chromatin states control cellular memory and differentiation in animals and plants (Roh *et al*, 2006; Zhang, 2008). Thus, we hypothesized that primed genes could be poised for enhanced activation of gene expression by histone modifications. To identify potential target genes of priming, we tested 11 *Arabidopsis* genes encoding WRKY transcription factors (*WRKY6, WRKY11, WRKY18, WRKY22, WRKY23, WRKY26, WRKY29, WRKY31, WRKY48, WRKY53* and *WRKY66*) for gene priming after BTH application (data not shown). BTH was chosen as the elicitor of priming because it induces gene priming at moderate concentrations (100–300 μM; Kohler *et al*, 2002; Beckers *et al*, 2009). *WRKY29, WRKY6* and *WRKY53* showed a typical priming response in expression (Fig 1A–C); application of the priming agent BTH alone did not activate *WRKY29*, and only activated *WRKY6* and *WRKY53* to a limited extent. Similar levels of gene expression were observed when plants were stressed by infiltration of water into their leaves. This has previously been used as a challenging stress (Kohler *et al*, 2002; Beckers *et al*, 2009) because it elicits a cell collapse or wound stress response in the entire leaf

that is more uniform than, for example, bacterial infection. Water infiltration after BTH treatment resulted in strongly enhanced gene activation, compared with plants that were stressed without previous BTH treatment (Fig 1A–C).

From the same samples, by using chromatin immunoprecipitation we analysed methylation of histone H3 Lys 4 (H3K4me) and acetylation of several lysine residues on histones H3 and H4 (H3ac, H4ac) on the promoters of the selected *WRKY* genes. The specificity of the chromatin immunoprecipitation reaction was evaluated in advance by measuring histone modifications on genes that were known to be transcriptionally activated or suppressed by BTH treatment (supplementary Fig S1A,B online). On the *WRKY29* promoter (Fig 1D), trimethylation (H3K4me3) and dimethylation (H3K4me2) of H3K4 and all acetylations tested increased after BTH application although this did not induce *WRKY29* transcription (Fig 1A). Thus, chromatin marks normally associated with active genes (Pokholok *et al*, 2005) are set by the priming stimulus before gene activation. Particularly after previous priming, a stress stimulus enhanced some of the modifications—H3K4me3, H3K9ac and H4K12ac—on *WRKY29* (Fig 1D). For *WRKY6* and *WRKY53*, only minor changes in histone acetylation were observed after both priming and/or stress treatment (Fig 1E,F). However, for these genes, H3K4me3 was induced by BTH treatment alone, to levels that are otherwise only found on the fully active gene (BTH treatment plus subsequent stress exposure). Induction of H3K4me2 was stronger with BTH alone than with stress treatment, whereas H3K4me1 showed a reciprocal reduction (Fig 1E,F). Importantly, the enhancement of H3K4 trimethylation and dimethylation after BTH treatment was not caused by the concomitant gene induction (Fig 1B,C), as transcripts accumulated to higher levels after direct stress exposure. However, changes in histone trimethylation and dimethylation were weaker after stress application than they were after BTH treatment (Fig 1E,F). As an additional control, we measured transcript levels and histone modifications on the *Ubiquitin5* (*UBQ5*) gene (supplementary Fig S1C,D online). Transcript abundance was slightly reduced by stress treatment in the absence of BTH, concomitant with a decrease in H3K4me3 levels. All other modifications remained unchanged under these conditions. Moreover, nucleosome occupancy on the tested *WRKY* gene promoters was only slightly affected by the treatments (supplementary Fig S1E online). Together, these data imply that pre-stress application of BTH induces chromatin modifications on *WRKY* gene promoters that facilitate the activation of gene expression by subsequent stress. This might be due to increased accessibility of DNA in acetylated compared with non-acetylated chromatin (Eberharter & Becker, 2002; Kanno *et al*, 2004) or the provision of docking sites for gene activators by histone modifications (de la Cruz *et al*, 2005; Vermeulen *et al*, 2007).

We investigated whether histone modifications on *WRKY* gene promoters can be detected in leaves distal to localized foliar infection by the pathogen *Pseudomonas syringae* pv. *maculicola*. Localized *P. s. maculicola* infection primed the *WRKY* promoters in remote leaves for an augmented response to secondary stress (Fig 2A) and, furthermore, the transcriptional responses in distal leaves were similar to those observed with BTH (Fig 1). Our analysis of histone modifications focused on comparison between the primed and non-primed state and on modifications that were induced by BTH in the previous assay (Fig 2B). On the three

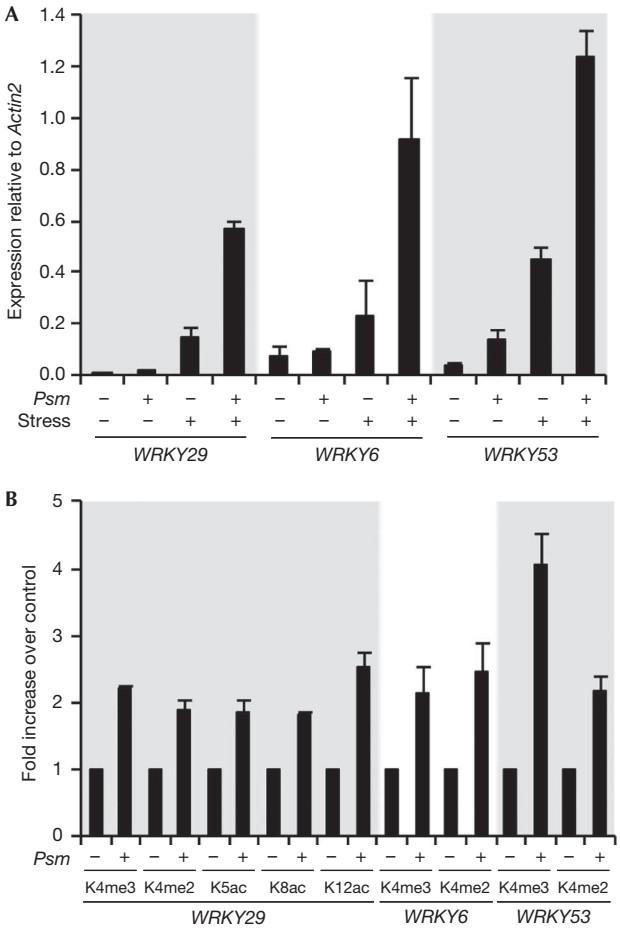

Fig 2 | Pathogen-induced priming for augmented gene activation. (**A**) Lower leaves were infected with *Psm*. After 72 h, upper leaves were left untreated or stressed by the infiltration of water. After 3 h, upper leaves were collected and analysed for transcript abundance. Data are standardized for abundance of the *Actin2* transcript. (**B**) Histone modifications in upper leaves 72 h after infection of lower leaves with *Psm*. Data are standardized for histone modification levels in the absence of pathogen infection. Each data point is based on at least three independent replicates. Error bars indicate s.e.m. values. ac, acetylation; me, methylation; *Psm*, *Pseudomonas syringae* pv. *maculicola*.

WRKY gene promoters, clear increases in histone modifications were observed after pathogen infection (Fig 2B). The response amplitude after perception of the systemic signals for SAR was similar to that observed after BTH treatment (Fig 1). Thus, pathogen exposure induces one or more systemic signals that are stored on gene promoters in remote leaves in the form of histone modifications.

Enhanced trimethylation of H3K4 concomitant with gene priming is a common feature of the assayed *WRKY* promoters. Next, we measured this histone modification in mutants that are attenuated in gene priming (*npr1*) or show permanent priming (*cpr1*, *edr1*) and constitutive pathogen resistance (*sni1*; see Introduction).

The transcriptional response of *WRKY29* to BTH and stress treatment is shown in Fig 3A. In the *npr1* mutant, *WRKY29* was responsive to stress treatment, but this response was not

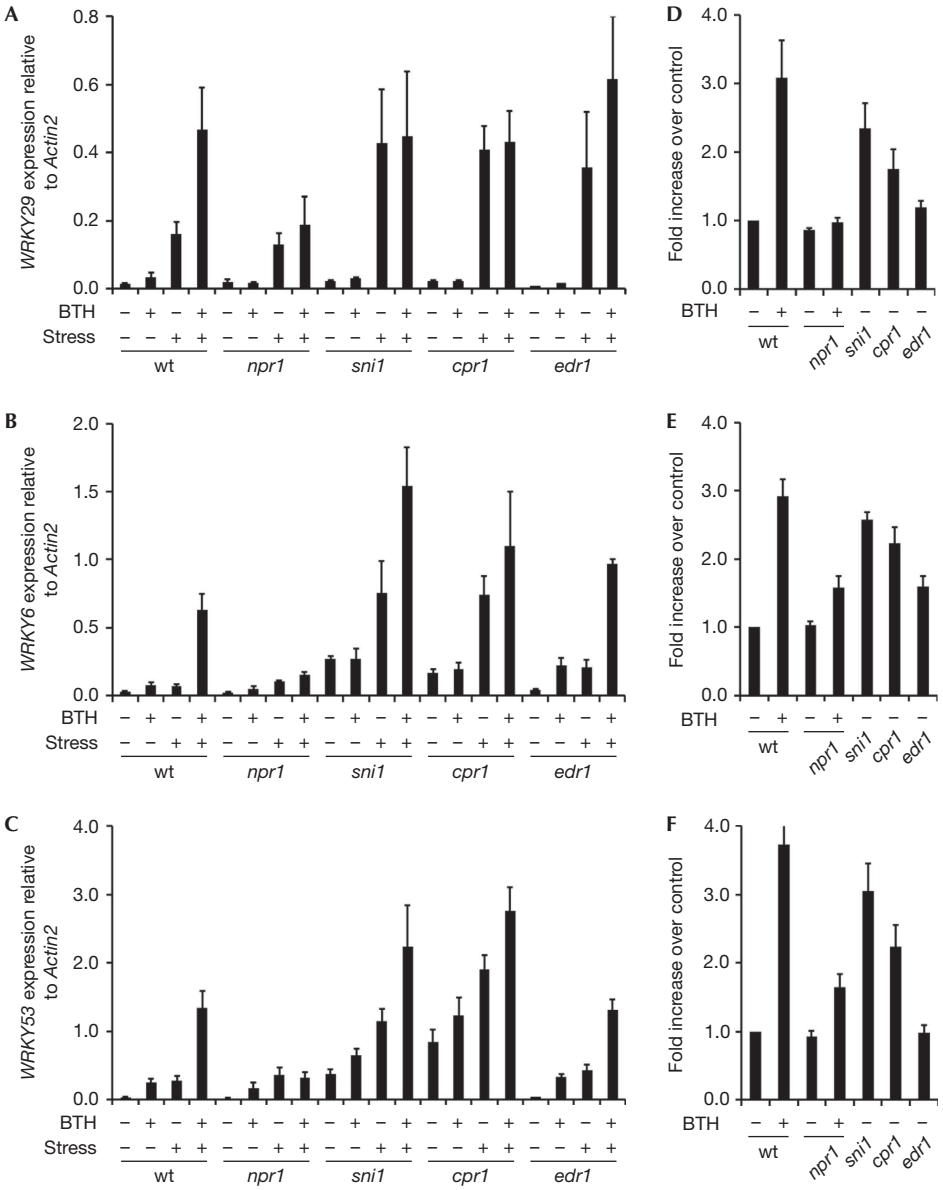

Fig 3 | Potentiated gene activation and H3K4 trimethylation in *npr1*, *sni1*, *cpr1* and *edr1* mutants. Wild-type and mutant plants were treated with 100 μM BTH or wettable powder (control). After 72 h, some of the plants were additionally stressed by infiltrating water into their leaves. Three hours later, leaves were collected and RNA and chromatin were extracted. (**A–C**) Transcript abundance in wild-type and mutant plants as determined by RT–qPCR. Data are standardized for abundance of the *Actin2* transcript. (**D–F**) Histone H3 Lys 4 trimethylation on gene promoters in wild-type and mutant plants. Data are standardized for wild-type histone modification levels in the absence of BTH. Data represent at least three independent replicates. Error bars indicate s.e.m. values. BTH, acibenzolar *S*-methyl; RT–qPCR, reverse transcriptase–quantitative PCR; wt, wild type.

augmented by earlier BTH application. By contrast, in the *sni1*, *cpr1* and *edr1* mutants, BTH treatment was not required for the strongest *WRKY29* activation in response to stress exposure. Transcription levels detected in these mutants in the absence of BTH were similar to those observed in the stress-exposed wild type after priming with BTH. This indicates that *WRKY29* was already primed in these mutants, in the absence of the inducer. Consistent with the transcriptional response, BTH induced trimethylation of H3K4 on the *WRKY29* promoter in the wild type, but not on the priming-deficient *npr1* mutant (Fig 3D). In the constitutively primed *sni1* and *cpr1* mutants (Fig 3A), H3K4me3 levels were already enhanced in the absence of BTH pretreatment. However, this was not found for the *edr1* mutant in which H3K4me3 levels were low.

In the assayed mutants, the results were similar for *WRKY6* and *WRKY53* expression and histone modifications. Neither gene showed augmented expression after BTH pretreatment and stress stimulus in the *npr1* mutant (Fig 3B,C). This correlated with the impaired ability of *npr1* to induce high H3K4me3 levels on the *WRKY6* and *WRKY53* promoters in response to BTH (Fig 3E,F).

In the *sni1* and *cpr1* mutants, the basal response to stress was augmented to levels normally observed in wild-type plants only after priming by BTH, although some additional induction of transcription was observed when the mutants were pretreated with BTH. For the *WRKY6* and *WRKY53* promoters, constitutively high H3K4me3 levels were detected in *sni1* and *cpr1* (Fig 3E,F). In the *edr1* mutant, the transcriptional response of *WRKY6* and *WRKY53* to BTH application and stress treatment was similar to the pattern found in the wild-type, indicating that the genes were not strongly primed in this mutant. Consequently, compared with the wild type, enhancement of basal H3K4me3 levels was almost undetectable (*WRKY6*) or absent (*WRKY53*). Together, our mutant analyses link H3K4 trimethylation as a molecular footprint to gene priming as the functional outcome. Whereas the association between H3K4me3 modification and gene priming is given in *npr1*, *sni1* and *cpr1*, constitutive priming of *WRKY29* in *edr1* does not seem to require high H3K4me3 levels. This might indicate the presence of a second independent process controlling priming in this mutant. Alternatively, weak or transient changes in histone modification might not have been detected in our experiments.

Not many examples exist that correlate histone modifications with a transcriptionally poised state. In maize, the tissue specificity of photosynthetic genes is controlled by H3K4me3 and is established independently of transcriptional activation (Offermann *et al*, 2006; Danker *et al*, 2008; Horst *et al*, 2009). Similar stimulus-dependent control of histone modifications was described for the vernalization response in *Arabidopsis* (He & Amasino, 2005) and the hormonal regulation of the *beta-phaseolin* promoter in beans (Ng *et al*, 2006). A genome-wide study in human cells revealed that about half of the inactive genes have nucleosomes that carry H3K4me3 or histone acetylations (Guenther *et al*, 2007). In our study, the abundance of H3K4me2 on primed genes before stress treatment (Figs 1D–F, 2B and 3B,C) is intriguing. H3K4me2 often colocalizes with H3K4me3 in vertebrates (Ruthenburg *et al*, 2007), but H3K4me2 has also been associated with poised states of genes in yeast and vertebrates (Pokholok *et al*, 2005; Bernstein *et al*, 2006). As the WD repeat-containing protein 5 component of the human methyltransferase complex preferentially binds to histone H3 when dimethylated at Lys 4 (Wysocka *et al*, 2005), high levels of H3K4me2 might speed-up or enhance subsequent trimethylation, facilitating the recruitment of chromatin remodelling factors and other effector proteins (Wysocka *et al*, 2006; Ruthenburg *et al*, 2007). As gene priming is part of the induced immune response in plants (Conrath, 2009) and animals (Chen *et al*, 1992; Pham *et al*, 2007), it will be interesting to see whether pre-stress modification of chromatin on defence gene promoters also has a function in animal defence.

METHODS

A. thaliana (accession Columbia-0) wild-type plants and *npr1*, *sni1*, *cpr1* and *edr1* mutants were grown in short day conditions (8 h light, 100 μmol m^{-2} s^{-1}) at 20 °C in a growth chamber. Treatments with wettable powder or 100 μM BTH and water infiltration were as described previously (Beckers *et al*, 2009). For pathogen-induced priming, three lower leaves were infiltrated with a suspension of *P. s. maculicola* (5×10^5 colony-forming units per millilitre).

RNA was isolated from leaves by using the TRIZOL method (Chomczynski, 1993). Transcript abundance was measured by reverse transcriptase–quantitative PCR on an ABI Prism 7300 sequence detector system (Applied Biosystems) using gene-specific primers (supplementary Table S1 online) and SYBR Green fluorescence (Platinum SYBR Green qPCR Mix, Invitrogen) for detection. Data were standardized for *Actin2* transcript abundance.

Chromatin isolation and immunoprecipitation were performed as described previously (Haring *et al*, 2007). The antibodies used for precipitation of modified histones from 2 g of leaf material are listed in supplementary Table S2 online. The abundance of DNA sequences in the precipitate was measured by quantitative PCR using the primers listed in supplementary Table S1 online. Background signals with serum derived from rabbits that were immunized with an unrelated potato protein never exceeded 10% of positive signals.

Supplementary information is available at EMBO *reports* online (http://www.emboreports.org).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Asai T, Tena G, Plotnikova J, Willmann MR, Chiu WL, Gomez-Gomez L, Boller T, Ausubel FM, Sheen J (2002) MAP kinase signalling cascade in *Arabidopsis* innate immunity. *Nature* **415:** 977–983

Beckers GJ, Jaskiewicz M, Liu Y, Underwood WR, He SY, Zhang S, Conrath U (2009) Mitogen-activated protein kinases 3 and 6 are required for full priming of stress responses in *Arabidopsis thaliana*. *Plant Cell* **21:** 944–953

Bernstein BE *et al* (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* **125:** 315–326

Chen TY, Lei MG, Suzuki T, Morrison DC (1992) Lipopolysaccharide receptors and signal transduction pathways in mononuclear phagocytes. *Curr Top Micro Immunol* **181:** 169–188

Chomczynski P (1993) A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. *Biotechniques* **15:** 532–534

Conrath U (2009) Priming of induced plant defense responses. *Adv Bot Res* **51:** 361–395

Danker T, Dreesen B, Offermann S, Horst I, Peterhansel C (2008) Developmental information but not promoter activity controls the methylation state of histone H3 lysine 4 on two photosynthetic genes in maize. *Plant J* **53:** 465–474

de la Cruz X, Lois S, Sanchez-Molina S, Martinez-Balbas MA (2005) Do protein motifs read the histone code? *Bioessays* **27:** 164–175

Dong J, Chen C, Chen Z (2003) Expression profiles of the *Arabidopsis* WRKY gene superfamily during plant defense response. *Plant Mol Biol* **51:** 21–37

Durrant WE, Dong X (2004) Systemic acquired resistance. *Annu Rev Phytopathol* **42:** 185–209

Eberharter A, Becker PB (2002) Histone acetylation: a switch between repressive and permissive chromatin. *EMBO Rep* **3:** 224–229

Frye CA, Innes RW (1998) An *Arabidopsis* mutant with enhanced resistance to powdery mildew. *Plant Cell* **10:** 947–956

Frye CA, Tang D, Innes RW (2001) Negative regulation of defense responses in plants by a conserved MAPKK kinase. *Proc Natl Acad Sci USA* **98:** 373–378

Garcia-Ramirez M, Rocchini C, Ausio J (1995) Modulation of chromatin folding by histone acetylation. *J Biol Chem* **270:** 17923–17928

Guenther MG, Levine SS, Boyer LA, Jaenisch R, Young RA (2007) A chromatin landmark and transcription initiation at most promoters in human cells. *Cell* **130:** 77–88

Haring M, Offermann S, Danker T, Horst I, Peterhansel C, Stam M (2007) Chromatin immunoprecipitation: quantitative analysis and data normalization. *Plant Methods* **2:** 11

Hayes MP, Wang J, Norcross MA (1995) Regulation of interleukin-12 expression in human monocytes: selective priming by interferon-gamma of lipopolysaccharide-inducible p35 and p40 genes. *Blood* **86:** 646–650

He Y, Amasino RM (2005) Role of chromatin modification in flowering-time control. *Trends Plant Sci* **10:** 30–35

Horst I, Offermann S, Dreesen B, Niessen M, Peterhansel C (2009) Core promoter acetylation is not required for high transcription from the phosphoenolpyruvate carboxylase promoter in maize. *Epigenet Chromatin* **2:** 17

Kanno T, Kanno Y, Siegel RM, Jang MK, Lenardo MJ, Ozato K (2004) Selective recognition of acetylated histones by bromodomain proteins visualized in living cells. *Mol Cell* **13:** 33–43

Kohler A, Schwindling S, Conrath U (2002) Benzothiadiazole-induced priming for potentiated responses to pathogen infection, wounding, and infiltration of water into leaves requires the *NPR1*/*NIM1* gene in *Arabidopsis*. *Plant Physiol* **128:** 1046–1056

Luger K, Mäder AW, Richmond RK, Sargent DF, Richmond TJ (1997) Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* **389:** 251–260

Maleck K, Levine A, Eulgem T, Morgan A, Schmid J, Lawton KA, Dangl JL, Dietrich RA (2000) The transcriptome of *Arabidopsis thaliana* during systemic acquired resistance. *Nat Genet* **26:** 403–410

March-Díaz R, García-Domínguez M, Lozano-Juste J, León J, Florencio FJ, Reyes JC (2008) Histone H2A.Z and homologues of components of the SWR1 complex are required to control immunity in *Arabidopsis*. *Plant J* **53:** 475–487

Mosher RA, Durrant WE, Wang D, Song J, Dong X (2006) A comprehensive structure-function analysis of *Arabidopsis* SNI1 defines essential regions and transcriptional repressor activity. *Plant Cell* **18:** 1750–1765

Ng DW, Chandrasekharan MB, Hall TC (2006) Ordered histone modifications are associated with transcriptional poising and activation of the phaseolin promoter. *Plant Cell* **18:** 119–132

Offermann S, Danker T, Dreymüller D, Kalamajka R, Töpsch S, Weyand K, Peterhänsel C (2006) Illumination is necessary and sufficient to induce histone acetylation independent of transcriptional activity at the C_4-specific phosphoenolpyruvate carboxylase promoter in maize. *Plant Physiol* **141:** 1078–1088

Pham LN, Dionne MS, Shirasu-Hiza M, Schneider DS (2007) A specific primed immune response in *Drosophila* is dependent on phagocytes. *PLoS Pathog* **3:** e26

Pokholok DK *et al* (2005) Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell* **122:** 517–527

Roh TY, Cuddapah S, Cui K, Zhao K (2006) The genomic landscape of histone modifications in human T cells. *Proc Natl Acad Sci USA* **103:** 15782–15787

Rushton PJ, Somssich IE, Ringler P, Shen QJ (2010) WRKY transcription factors. *Trends Plant Sci* **15:** 247–258

Ruthenburg AJ, Allis CD, Wysocka J (2007) Methylation of lysine 4 on histone H3: intricacy of writing and reading a single epigenetic mark. *Mol Cell* **25:** 15–30

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner HY, Hunt MD (1996) Systemic acquired resistance. *Plant Cell* **8:** 1809–1819

Senaratna T, Touchell D, Bunn E, Dixon K (2000) Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. *Plant Growth Reg* **30:** 157–161

van den Burg HA, Takken FLW (2009) Does chromatin remodeling mark systemic acquired resistance? *Trends Plant Sci* **14:** 286–294

Vermeulen M, Mulder KW, Denissov S, Pijnappel WW, van Schaik FM, Varier RA, Baltissen MP, Stunnenberg HG, Mann M, Timmers HT (2007) Selective anchoring of TFIID to nucleosomes by trimethylation of histone H3 lysine 4. *Cell* **131:** 58–69

Wysocka J, Swigut T, Milne TA, Dou Y, Zhang X, Burlingame AL, Roeder RG, Brivanlou AH, Allis CD (2005) WDR5 associates with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development. *Cell* **121:** 859–872

Wysocka J *et al* (2006) A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. *Nature* **442:** 86–90

Zhang X (2008) The epigenetic landscape of plants. *Science* **320:** 489–492

Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors

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Salicylic acid (SA) is a defense hormone required for both local and systemic acquired resistance (SAR) in plants. Pathogen infections induce SA synthesis through up-regulating the expression of *Isochorismate Synthase 1* (*ICS1*), which encodes a key enzyme in SA production. Here we report that both SAR Deficient 1 (SARD1) and CBP60g are key regulators for *ICS1* induction and SA synthesis. Whereas knocking out *SARD1* compromises basal resistance and SAR, overexpression of *SARD1* constitutively activates defense responses. In the *sard1-1 cbp60g-1* double mutant, pathogen-induced *ICS1* up-regulation and SA synthesis are blocked in both local and systemic leaves, resulting in compromised basal resistance and loss of SAR. Electrophoretic mobility shift assays showed that SARD1 and CBP60g represent a plant-specific family of DNA-binding proteins. Both proteins are recruited to the promoter of *ICS1* in response to pathogen infections, suggesting that they control SA synthesis by regulating *ICS1* at the transcriptional level.

plant immunity | SAR Deficient 1 | Isochorismate Synthase 1 | CBP60g

Systemic acquired resistance (SAR) is a secondary immune response in the distal parts of plants activated by local defense responses. SAR is long-lasting and effective against a broad spectrum of pathogens, including fungi, bacteria, and viruses (1). Traditionally, SAR is induced by incompatible pathogens that cause localized cell death. Tissue necrosis at inoculation sites is not required for SAR activation, however (2).

Salicylic acid (SA) is a phytohormone that plays a central role in defense signaling (3). It is required for both basal defense and SAR. Early studies showed that pathogen infections lead to increased SA levels in both local and distal parts of plants (4–6). Whereas application of exogenous SA or SA analogs induces resistance to pathogens (7–9), degradation of SA by transforming plants with the bacterial salicylate hydroxylase gene *NahG* blocks SA accumulation and SAR (10). SA activates defense responses through its downstream components NPR1 (11) and three redundant transcription factors, TGA2, TGA5, and TGA6 (12). Increased SA levels induce redox changes and result in reduction of NPR1 to a monomeric form that accumulates in the nucleus to activate defense gene expression (13).

In *Arabidopsis*, mutations in *SID2* and *EDS5* block pathogen-induced SA synthesis and result in defects in SAR as well as basal resistance (14). *SID2* encodes Isochorismate Synthase 1 (ICS1), a key enzyme in pathogen-induced SA biosynthesis (15). ETHYLENE INSENSITIVE3 (EIN3) and EIN3-LIKE1 have been reported to negatively regulate SA synthesis through repression of *ICS1* expression (16). How the *ICS1* expression is positively regulated during pathogen infection remains to be determined. Because up-regulation of SA biosynthesis is critical to the activation of basal resistance and SAR, identification of upstream regulatory components required for the induction of SA biosynthesis genes such as *ICS1* is essential for understanding SA-mediated defense responses. Here we report our discovery of two members of a plant-specific family of transcription factors that regulate the induction of *ICS1* and the accumulation of SA on pathogen infection.

Results

Development of an SAR Assay. In traditional SAR assays for *Arabidopsis*, local leaves are infiltrated with avirulent bacteria to induce SAR, and distal leaves are later challenged with a virulent bacterial pathogen. Bacterial growth in the distal leaves is quantified to determine whether SAR indiction occurred. Because quantifying bacterial growth for a large number of plants is tedious and bacterial growth varies depending on the growth conditions, traditional SAR assays are unsuitable for large-scale screening of SAR-deficient mutants.

To address this problem, we tested whether SAR can be induced against the oomycete pathogen *Hyaloperonospora arabidopsidis* Noco2 (*H.a.* Noco2) after infiltration with *Pseudomonas syringae* p.v. maculicola (*P.s.m.*) ES4326. We found that infiltrating local leaves with a low dose of *P.s.m.* ES4326 (OD_{600} = 0.001) consistently induced SAR against *H.a.* Noco2 (Fig. 1*A*). We then tested a selection of known SAR mutants using this assay. As shown in Fig. 1*B*, SAR was severely compromised in all mutants tested, including *sid2-1*, *eds5-3*, *npr1-1*, *eds1-2* (Col), and *pad4-1*. Because this assay is easy to perform and provides consistent results, it is feasible for application in a large number of plants.

***SARD1* Is Required for SAR.** To identify genes required for SAR, we assayed T-DNA insertion mutants of ~200 genes induced by *P.s.m.* ES4326 for loss of the SAR phenotype. These genes and their corresponding T-DNA mutants have been described previously (17). Among them, only *At1g73805* was found to be required for SAR. Both SALK_138476 and SALK_052422 contain T-DNAs in the second intron of *At1g73805* (Fig. 2*A*), which disrupt expression of the gene (Fig. 2*B*). On primary induction with *P.s.m.* ES4326, WT plants developed systemic resistance to *H.a.* Noco2. In contrast, SAR was reduced in both mutants and was lost in the *npr1-1* control plants (Fig. 2*C*). Given the SAR-deficient phenotypes, we designated *At1g73805* as *SAR Deficient 1* (*SARD1*), SALK_138476

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The authors declare no conflict of interest.

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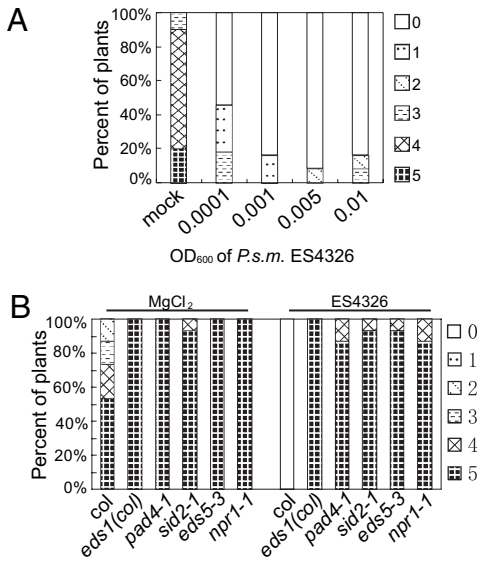

Fig. 1. Growth of *H.a.* Noco2 on the distal leaves of WT and SAR-deficient mutants. (*A*) Induction of SAR by infiltrating two primary leaves of 3-wk-old plants with different concentrations (OD_{600}) of *P.s.m.* ES4326. (*B*) Testing of SAR response in known SAR-deficient mutants. Two days after two primary leaves were infiltrated with *P.s.m.* ES4326 (OD_{600} = 0.001) or 10 mM $MgCl_2$ (mock), plants were sprayed with *H.a.* Noco2 spores at a concentration of 5×10^4 spores per mL of water. Infection was scored 7 d later by counting the number of conidiophores on the distal leaves. A total of 15 plants were scored for each treatment. Disease rating scores are as follows: 0, no conidiophores on the plants; 1, one leaf infected with no more than five conidiophores; 2, one leaf infected with more than five conidiophores; 3, two leaves infected, but with no more than five conidiophores on each infected leaf; 4, two leaves infected with more than five conidiophores on each infected leaf; 5, more than two leaves infected with more than five conidiophores.

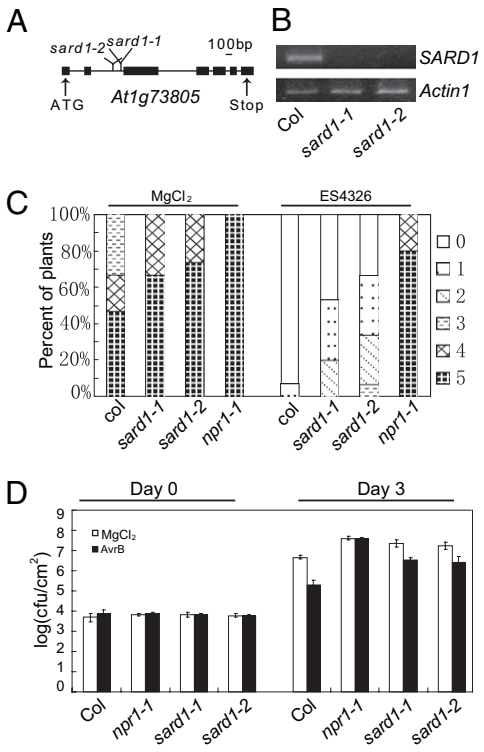

Fig. 2. Analysis of *sard1* knockout mutants. (*A*) Positions of T-DNA insertions within *SARD1*. *sard1-1*, SALK_138476; *sard1-2*, SALK_052422. Lines indicate introns, and black boxes are exons. (*B*) RT-PCR analysis of *SARD1* expression in *sard1-1* and *sard1-2*. (*C*) Growth of *H.a.* Noco2 on systemic leaves of the indicated genotypes. Three-wk-old plants were first infiltrated with *P.s.m.* ES4326 (OD_{600} = 0.001) or 10 mM $MgCl_2$ on two primary leaves and sprayed with *H.a.* Noco2 spores 2 d later. At 7 d postinoculation, infections were scored as described in Fig. 1. (*D*) Growth of *P.s.m.* ES4326 on systemic leaves of the indicated genotypes. Two leaves from each plant were infiltrated with *P.s.m.* ES4326 AvrB (OD_{600} = 0.02) or 10 mM $MgCl_2$ 2 d before *P.s.m.* ES4326 infection (OD_{600} = 0.001) on distal leaves. The bacterial titers were measured by taking leaf discs within the inoculated area. Error bars represent 95% confidence limits of log-transformed data from four replicates.

as *sard1-1*, and SALK_052422 as *sard1-2*. As shown in Fig. S1 *A* and *B*, *P.s.m.* ES4326 induced the expression of *SARD1* in both local and systemic leaves.

We then tested whether SAR can be induced in the *sard1* mutants in a traditional SAR assay. As shown in Fig. 2*D*, *P.s.m.* ES4326 carrying *AvrB* induced systemic resistance to *P.s.m.* ES4326 in the WT plants, whereas SAR responses were partly compromised in *sard1-1* and *sard1-2*. Taken together, these results indicate that SARD1 functions as a positive regulator of SAR.

Overexpression of *SARD1* Leads to Enhanced Resistance to Pathogens. When *SARD1* with or without a C-terminal HA tag was expressed under the control of its native promoter in WT plants, about one-quarter of T1 transgenic plants were dwarfed, suggesting possible activation of defense responses in these plants. Two representative *SARD1-HA* lines were characterized in detail. Lines 1 and 2 expressed *SARD1-HA* at different levels, with higher expression in line 1 than in line 2 (Fig. 3*A*). As shown in Fig. S1*E*, line 1 was smaller than WT and line 2, and senesced early. No spontaneous lesion formation was observed in these lines. The greater expression of SARD1-HA in line 1 was confirmed by Western blot analysis (Fig. S1*F*). Analysis of defense marker genes *PR1* (Fig. 3*B*) and *PR2* (Fig. 3*C*) showed that both lines exhibit constitutive expression of the *PR* genes, with greater expression of *PR1* and *PR2* in line 1. Furthermore, line 1 displayed strongly enhanced resistance to *H.a.* Noco2 (Fig. 3*D*). Enhanced resistance to *P.s.m.* ES4326 was also observed in line 1 (Fig. S1*G*), suggesting that overexpression of *SARD1* leads to enhanced resistance to pathogens. Analysis of SA levels showed that greater accumulation of both free and total SA in line 1 compared with WT and line 2 (Fig. 3 *E* and *F*).

Mutations in *SARD1* and *CBP60g* Have Additive Effects on SAR. SARD1 belongs to a plant-specific protein family previously termed ACBP60 (Fig. S2) (18). We found that knocking out another member of the ACBP60 family, CBP60g, has a small but reproducible effect on SAR. Like *SARD1*, *CBP60g* is also induced by *P.s.m.* ES4326 in both local and systemic leaves (Fig. S1 *C* and *D*). Because SARD1 and CBP60g belong to the same protein family, we generated the *sard1-1 cbp60g-1* double mutant to test whether these proteins have additive effects on SAR. The double mutant displayed WT morphology (Fig. S3*A*). As shown in Fig. 4*A* , systemic resistance to *H.a.* Noco2 induced by *P.s.m.* ES4326 was further impaired in the double mutant. In addition, systemic resistance to *P.s.m.* ES4326 induced by *P.s.m.* ES4326 *avrB* was also lost in the double mutant (Fig. S3*B*). These data suggest that SARD1 and CBP60g have overlapping functions or function in two independent pathways in the regulation of SAR.

To test whether SARD1 and CBP60g are also required for basal defense, we inoculated the single and double mutants with *P.s.m.* ES4326. As shown in Fig. 4*B*, compared with WT, *sard 1-1* supported about 10-fold more bacterial growth, whereas *cbp60g-1* exhibited only slightly greater bacterial growth. Bacterial growth was much

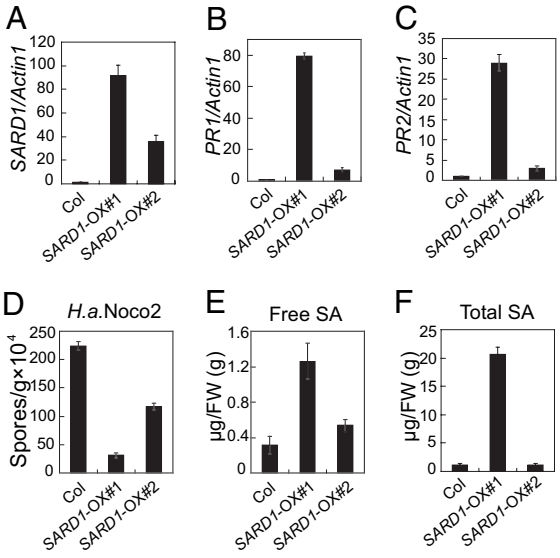

Fig. 3. Overexpression of *SARD1* leads to constitutive activation of defense responses and increased SA levels. (*A*) *SARD1* expression in two independent *SARD1-HA* transgenic lines. (*B* and *C*) Expression of *PR1* (*B*) and *PR2* (*C*) in the indicated genotypes. (*D*) Growth of *H.a.* Noco2 on the indicated genotypes. The plants were sprayed with *H.a.* Noco2 spores at a concentration of 5×10^4 spores/mL of water. Infection was scored at 7 d postinoculation. (*E* and *F*) Free SA (*E*) and total SA (*F*) levels in the indicated genotypes.

greater in *sard1-1 cbp60g-1* than in the single mutants, suggesting that SARD1 and CBP60 also play additive roles in basal defense.

SARD1 and CBP60g are required for SA accumulation and induction of *ICS1* during pathogen infection. The loss of SAR phenotype in *sard1-1 cbp60g-1* prompted us to test whether SA biosynthesis is affected in the mutant plants. As shown in Fig. 5 *A* and *B*, SA levels were similar in the WT and the mutants before induction. After induction by *P.s.m.* ES4326 *avrB*, SA levels increased in the WT and the single mutants, but not in the double mutant, indicating blockage of pathogen-induced SA accumulation in the double mutant.

In plants, pathogen-induced SA is synthesized from chorismate by ICS1. *ICS1* expression is induced by both biotic and abiotic stresses. To test whether induction of *ICS1* is affected in *sard1-1 cbp60g-1*, we analyzed the expression of *ICS1* before and after induction by UV-B irradiation or bacterial infection. As shown in Fig. 5 *C* and *D*, induction of *ICS1* was blocked in the double mutant, suggesting that the reduced SA synthesis in the double mutant is caused by loss of *ICS1* induction. Further analysis revealed that induction of *ICS1* and SA synthesis by *P.s.m.* ES4326 *avrB* in the systemic leaves was also blocked in the double mutant (Fig. 5 *E* and *F*).

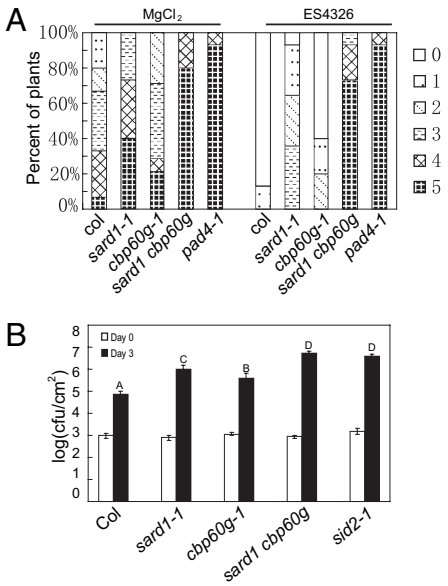

Fig. 4. Loss of SAR and basal resistance in *sard1-1 cbp60g-1*. (*A*) Growth of *H.a.* Noco2 on systemic leaves of the indicated genotypes. Primary infection with *P.s.m.* ES4326, secondary inoculation with *H.a.* Noco2, and scoring of the infection were performed as shown in Fig. 2*C*. (*B*) Growth of *P.s.m.* ES4326 on the indicated genotypes. Leaves of 5-wk-old plants were infiltrated with a bacterial suspension at OD_{600} = 0.0002. Bacterial titers were measured on day 0 and day 3. The values presented are averages of six replicates ± SD. Statistically significant differences among the samples are labeled with different letters ($P < 0.01$).

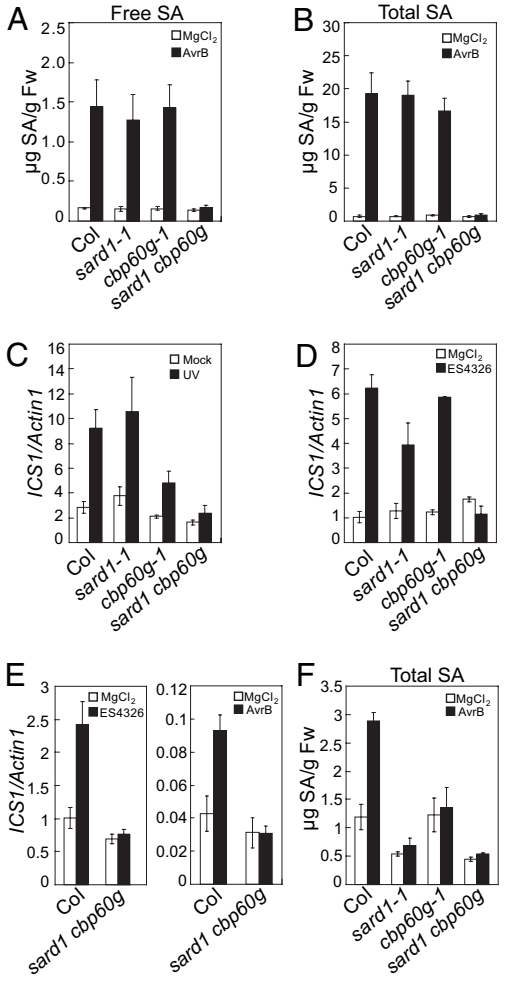

Fig. 5. *ICS1* induction and SA accumulation are blocked in *sard1-1 cbp60g-1*. (*A* and *B*) Induction of free SA (*A*) and total SA (*B*) synthesis by *P.s.m.* ES4326 *AvrB*. Plants were infiltrated with *P.s.m.* ES4326 AvrB (OD_{600}= 0.2), and the inoculated leaves were collected 48 h later for SA extraction. (*C*) Induction of *ICS1* by UV-B in the indicated genotypes. Plants were treated by UV-B for 15 min, and samples were taken 24 h postirradiation. (*D* and *E*) Induction of *ICS1* in local (*D*) and systemic leaves (*E*) of the indicated genotypes. Plants were infiltrated with *P.s.m.* ES4326 (OD_{600}= 0.001) or *P.s.m.* ES4326 *avrB* (OD_{600}= 0.01). Samples were taken 48 h postinoculation. (*F*) SA accumulation in the systemic leaves after local infections. Three leaves of 4-wk-old plants were infiltrated with *P.s.m.* ES4326 *avrB* (OD_{600}= 0.01), and the distal leaves were collected 48 h later for total SA extraction.

SARD1 Is Targeted to the Promoter of *ICS1* After Pathogen Infection. To determine the subcellular localization of SARD1, we generated transgenic plants expressing the SARD1-GFP fusion protein under its own promoter. Some of the transgenic lines exhibited small stature like the *SARD1-HA* overexpression lines described earlier (Fig. S4*A*), suggesting that SARD1-GFP functions similar to SARD1. SARD1-GFP was found to localize in the nucleus of leaf pavement cells after infiltration with *P.s.m.* ES4326 (Fig. 6*A*). Without induction, no green fluorescence was observed in the *SARD1-GFP* transgenic lines, likely due to low levels of the protein. Localization of SARD1 to the nucleus was further confirmed by fractionation and Western blot analysis of protein extracts from *SARD1-HA* transgenic plants (Fig. S4*B*).

The requirement for SARD1 and CBP60 for the expression of *ICS1* and the nuclear localization of SARD1 prompted us to test whether these proteins are recruited to the promoter of *ICS1*. We carried out chromatin immunoprecipitation (ChIP) on *SARD1-HA* transgenic plants using anti-HA antibody, and used real-time PCR on the immunoprecipitated DNA to test whether genomic DNA in the promoter region of *ICS1* was enriched by ChIP. Without induction, no enrichment was observed. Induction by *P.s.m.* ES4326 or UV-B led to enrichment of DNA around the *ICS1* promoter (Fig. 6*B* and Fig. S4*C*), suggesting that SARD1 is targeted to the *ICS1* promoter. ChIP analysis with *CBP60g-HA* transgenic plants showed that CBP60g was also recruited to the *ICS1* promoter after induction by *P.s.m.* ES4326 (Fig. S4*D*).

To identify the region to which SARD1 is targeted on the *ICS1* promoter, we used 16 pairs of primers (Table S1) designed to amplify a group of overlapping DNA fragments covering the region from 2,351 bp upstream to 396 bp downstream of the translation start site of *ICS1* (Fig. 6*C*). As shown in Fig. 6*D*, DNA fragment 7 exhibited the greatest enrichment by ChIP compared with the other fragments, suggesting that SARD1 is targeted to this region after induction with UV-B. Similar results were obtained from ChIP analysis performed on *SARD1-HA* transgenic plants treated with *P.s.m.* ES4326 (Fig. 6*E*).

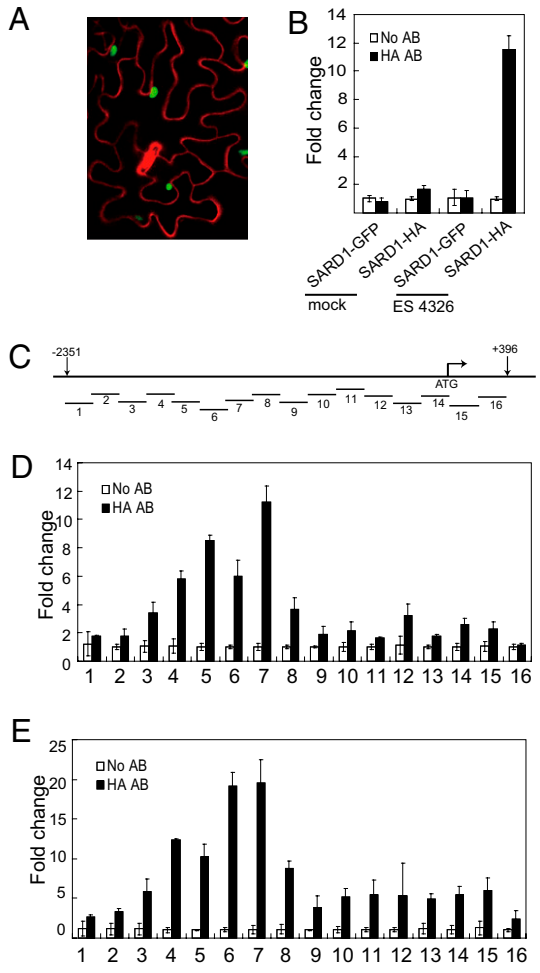

Fig. 6. ChIP analysis of recruitment of SARD1 to the promoter of *ICS1*. (*A*) GFP fluorescence in leaf pavement cells of *SARD1-GFP* transgenic plants expressing *SARD1-GFP* under its native promoter. Leaves were infiltrated with *P.s.m.* ES4326 (OD_{600}= 0.001) 24 h before being examined by confocal microscopy. Cell walls were stained with 5 mg/mL of propidium iodine. (*B*) *P.s.m.* ES4326–induced recruitment of SARD1 to the promoter of *ICS1*. (*C*) Locations of the PCR fragments. (*D*) Enrichment of SARD1-bound fragments of *ICS1* promoter after UV-B treatment. (*E*) Enrichment of SARD1-bound fragments of *ICS1* promoter after induction by *P.s.m.* ES4326. Plants were irradiated by UV-B for 15 min or infiltrated with *P.s.m.* ES4326 (OD_{600}= 0.001), and samples were taken 24 h later for ChIP analysis. ChIP was performed with an anti-HA antibody (AB) as described previously (24). Quantitative PCR was carried out using the immunoprecipitated DNA as a template. *SARD1-GFP* transgenic plants were used as negative controls in *B*.

SARD1 and CBP60g Are DNA-Binding Proteins. Five of the eight ACBP60 proteins were originally shown to bind calmodulin (CaM) (18). The CaM-binding motif is located at the C terminus of the proteins, but this motif is absent in SARD1 and CBP60g. A fragment of 76 amino acids at the N terminus of CBP60g was recently shown to bind CaM (19). To determine whether SARD1 is capable of binding CaM, we expressed full-length SARD1 and a fragment of SARD1 corresponding to the first 76 amino acids at the N terminus of CBP60g with a GST tag. As shown in Fig. S5, the N-terminal fragment of CBP60g bound to CaM, but no CaM-binding activity was detected with either the full-length or the truncated SARD1 protein. In CBP60g, amino acid Val-29 is required for the binding of CBP60g to CaM (19), and this residue is not conserved in SARD1 (Fig. S2), consistent with our finding that the N-terminal fragment of SARD1 was not able to bind CaM.

The proteins of the ACBP60 family all contain a highly conserved domain in their central region (Fig. S2). The function of this domain is unknown. Because SARD1 is localized to the nucleus and targeted to the promoter of *ICS1*, we tested whether it binds to DNA directly. Three overlapping fragments of SARD1 with C-terminal His-tags were expressed in *Escherichia coli* and purified using Ni-NTA columns. Electrophoretic mobility shift assays (EMSA) were subsequently carried out using the recombinant SARD1 proteins and DNA fragment 7 from the ChIP-PCR analysis (Fig. 6*C*). As shown in Fig. 7*A*, a mobility shift was observed when the ^{32}P-labeled DNA fragment was preincubated with the N-terminal half (aa 1–214), but not with the C-terminal half (aa 215–451), of SARD1. In addition to the indicated mobility shift, another shifted band close to the top of the gel was seen. Analysis of the SARD1 protein by gel filtration indicated that a large fraction of the protein was in an oligomerized form. The shifted band close to the top of the gel might represent oligomerized protein bound to the probe. Similar mobility shifts were observed when the DNA fragment was preincubated with the central region (aa 149–270) of SARD1. Binding of the SARD1 fragments to the labeled probe can be completed using an excess of unlabeled probe but not poly(dIdC) in the reaction, indicating that the binding that we observed is specific. These data suggest that SARD1 is a DNA-binding protein, and that the DNA-binding motif is in the region of aa 149–214.

To test whether CBP60g is also able to bind to DNA, we expressed the corresponding middle domain (aa 148–263) of CBP60g with a C-terminal His-tag in *E. coli*. The $CBP60g_{148–263}$ protein was purified using Ni-NTA columns and used in EMSA. As shown in Fig. S6*A*, $CBP60g_{148–263}$ was able to bind DNA fragment

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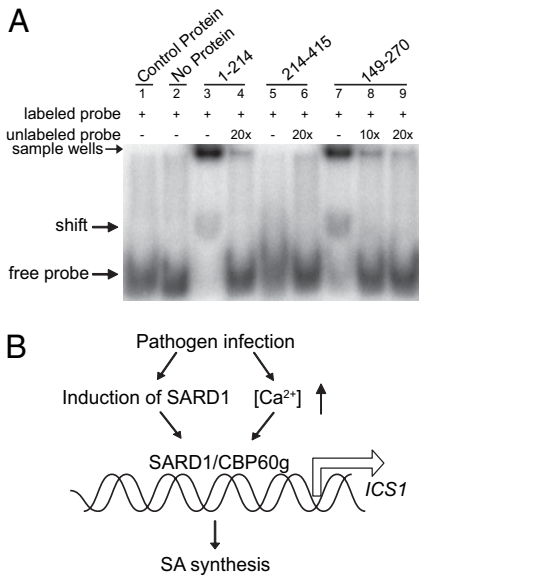

Fig. 7. EMSA analysis for binding of SARD1 to a DNA fragment from the *ICS1* promoter (*A*) and a working model for regulation of SA synthesis by SARD1 and CBP60g (*B*). (*A*) DNA fragment 7 from the ChIP-PCR analysis (Fig. 6) was end-labeled with [^{32}P]ATP. Then 0.1 pmol labeled DNA was incubated with 1.25 μg of *E. coli*–expressed truncated SARD1 proteins as indicated (lanes 3–9) or a control protein (the kinase domain of SOBIR1) preparation (lane 1), in the absence (lanes 1, 3, 5, and 7) or the presence (lanes 4, 6, 8, and 9) of unlabeled DNA fragment 7 as a cold competitor. The sample in lane 2 contains only the labeled probe. The 20× and 10× contain 20- and 10-fold more unlabeled probes than labeled probes, respectively. (*B*) A proposed model of two defense pathways for pathogen-induced SA synthesis. One pathway leads to induction of *SARD1* and subsequent recruitment of SARD1 to the promoter of *ICS1* and activation of its transcription and SA synthesis. The other pathway leads to Ca^{2+} influx, which results in binding of CaM to CBP60g and recruitment of CBP60g to the promoter of *ICS1* and activation of the transcription of *ICS1* and SA synthesis.

7 from the *ICS1* promoter, suggesting that CBP60g is also a DNA-binding protein.

To determine whether binding of CBP60g and SARD1 to DNA is sequence-specific, we synthesized 33 overlapping oligonucleotide probes (Table S2) covering the region of DNA fragment 7, and tested these probes for their relative binding affinities to $CBP60g_{148–263}$. One of the oligonucleotide probes (oligo-15; gaaattttgg) displayed relatively high affinity to the protein compared with other probes. As shown in Fig. S6*B*, binding of oligo-15 to $CBP60g_{148–263}$ can be competed by an excess of unlabeled oligo-15, but not by another oligonucleotide probe, oligo-8. In addition, binding of oligo-15 to the central domain of SARD1 can be efficiently competed by excess of unlabeled oligo-15, but not by oligo-8 (Fig. S6*C*). These data suggest that CBP60g and SARD1 are sequence-specific DNA-binding proteins.

Discussion

SA is one of the most important signal molecules for plant defense. Pathogen-induced SA synthesis and accumulation are required for both local resistance and SAR. How plants regulate SA biosynthesis is a fundamental question in plant immunity. Using a reverse genetic approach, we identified SARD1 as a key regulator of both SAR and basal defense. In the *sard1 cbp60g* double mutant, pathogen-induced SA synthesis in both local and systemic leaves is completely blocked, leading to severely compromised local and systemic resistance. Our data demonstrate that *SARD1* and *CBP60g* are crucial regulators for the induction of SA synthesis by pathogens.

Both SAR and local resistance are partially compromised in *sard1* and *cbp60g* single mutants. The compromised SAR likely results from reduced SA accumulation in the systemic leaves. The cause of the reduced local resistance is less clear. Whereas local SA levels are comparable in WT and the single mutants after infection by the avirulent pathogen *P.s.m.* ES4326 *avrB*, whether induction of SA synthesis by the virulent *P.s.m.* ES4326 is affected in the single mutants remains to be determined. It is possible that the compromised local resistance also results from reduced SA accumulation. Alternatively, reduced local resistance could be caused by a loss of induction of other genes regulated by SARD1 and CBP60g. Identifying additional target genes of SARD1 and CBP60g by ChIP sequencing will provide insight into the roles of SARD1 and CBP60g in local resistance.

SARD1 belongs to a protein family with unknown biochemical functions. One of the members, CBP60g, has previously been shown to contribute to MAMP-induced SA synthesis (19), but how CBP60g affects MAMP-induced SA synthesis remains unknown. We have shown that both SARD1 and CBP60g are recruited to the *ICS1* promoter in response to pathogen infections, suggesting that they directly regulate *ICS1* expression and SA synthesis. SARD1 is targeted to a 181-bp region on the *ICS1* promoter. This region contains a predicted W-box and a MYB recognition site. Using EMSA, we demonstrated that SARD1 and CBP60g are DNA-binding proteins. Both proteins preferentially bind the oligonucleotide probe oligo15 (gaaattttgg), which contains no known cis-acting element. Bioinformatic analysis using the microarray database at the Arabidopsis Resource Center showed that the "aatttt" motif on oligo15 is statistically overrepresented in the promoters of genes induced by flg22 or *Pseudomonas syringae pv. Tomato* (*P.s.t.*) DC3000 *avrRpm1* ($P < 10^{-5}$).

Binding of SARD1 and CBP60g to DNA is facilitated through the highly conserved central region of the proteins, which exhibits no sequence similarity to other known DNA-binding proteins, suggesting that they represent a plant-specific family of transcription factors. Because not all members in the ACBP family have CaM-binding activity but all share the central DNA-binding domain, we suggest renaming this protein family the SARD1 transcription factor family.

SARD1 shares only 39% identity with CBP60g at the amino acid level. The expression of both *SARD1* and *CBP60g* is up-regulated by pathogen infections. Whereas overexpression of *SARD1* leads to constitutive defense responses, similar activation of defense responses was not observed in transgenic plants overexpressing *CBP60g*. In CBP60g and SARD1, the middle domains that contain the DNA-binding activity are highly conserved, but sequences at the N- and C-termini are quite diverged. The N-terminal domains of CBP60g and SARD1 appear to have different functions. Whereas CBP60g binds to CaM through its N-terminal domain (19), SARD1 is not able to bind CaM. Activation of defense responses by overexpression of SARD1, but not of CBP60g, suggests that CBP60g, but not SARD1, requires activation by CaM.

Our data suggest that SA synthesis is activated through two parallel pathways (Fig. 7*B*), one dependent on SARD1 and the other dependent on CBP60g. Whereas the activity of CBP60g is most likely modulated by Ca^{2+}, activation *SARD1* at the transcription level by upstream regulators is sufficient to trigger downstream defense responses. One might ask why plants need two parallel pathways to activate SA synthesis. These two pathways might have evolved to respond to different stimuli. Another possibility is that plants use individually controlled pathways to fine-tune the timing and magnitude of SA synthesis for better control of SA-mediated defense responses.

In summary, we discovered two members of a plant-specific transcription factor family that regulate the expression of *ICS1* and SA synthesis. Separate dissection of the SARD1- and CBP60g-dependent pathways might provide a more complete picture of how

pathogen infections activate SA synthesis and suggest new strategies for engineering crop plants with improved pathogen resistance.

Materials and Methods

Plant Materials. The *sid2-1*, *eds5-3*, *npr1-1*, *eds1-2*, and *pad4-1* mutants used have been described previously (14, 20–22). Seeds of *sard1-1* (SALK_138476), *sard1-2* (SALK_052422), and *cbp60g-1* (SALK_023199) were obtained from the Arabidopsis Stock Center. Construction of the plasmids used for generating the transgenic plants is described in *SI Methods*.

Plant Growth Conditions and Mutant Analysis. Plants were grown at 23 °C under a 16-h light/8-h dark cycle in plant growth rooms or chambers. For infection with *H.a.* Noco2, spores at a concentration of 5×10^4 spores/mL were sprayed onto the plants, which were then maintained in a growth chamber with high humidity (>80%) at 18 °C under a 12-h light/12-h dark cycle for 1 wk. For gene expression analysis, RNA was extracted using Takara RNAiso reagent. Reverse transcription was carried out using the Takara M-MLV RTase cDNA synthesis kit. Real-time PCR was performed using Takara SYBR Premix Ex. The primers used to amplify *ICS1* were 5′-gaactcaaatctcaacctcc-3′ and 5′-actgcgacgagagaagaaac-3′. The primers used for amplification of *Actin1*, *PR-1*, and *PR-2* were described previously (12). SA was extracted and measured by HPLC as described previously (23).

EMSA. Expression and purification of the SARD1 and SARD2 proteins from *E. coli* is described in *SI Methods*. The 181-bp DNA fragment used in EMSA was amplified by PCR using primers 7F and 7R (Table S1). The probe was end-labeled by incubating 10 pmol of double-stranded DNA in a 40-μL reaction with 20 units of polynucleotide kinase (New England Biolabs) and 40 μCi of [γ-^{32}P]ATP. After labeling, the DNA was diluted to 100 μL total volume. Approximately 20 ng of the purified protein was mixed with 100 ng of poly[dI-dC], 1 μL of labeled probe (0.1 pmol per reaction), and 4 μL of 5× binding buffer [50 mM Hepes (pH 7.5), 375 mM KCl, 6.25 mM $MgCl_2$, 25% glycerol, 1 mM DTT) in a 20-μL reaction. The mixture was incubated on ice for 30 min and then run on a 4% (wt/vol) native polyacrylamide gel in 0.5× TGE buffer (12.5 mM Tris, 95 mM glycine, 0.5 mM EDTA; pH 8.8). The gel was dried and autoradiographed after electrophoresis.

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1. Durrant WE, Dong X (2004) Systemic acquired resistance. *Annu Rev Phytopathol* 42: 185–209.
2. Mishina TE, Zeier J (2007) Pathogen-associated molecular pattern recognition rather than development of tissue necrosis contributes to bacterial induction of systemic acquired resistance in *Arabidopsis*. *Plant J* 50:500–513.
3. Vlot AC, Dempsey DA, Klessig DF (2009) Salicylic acid, a multifaceted hormone to combat disease. *Annu Rev Phytopathol* 47:177–206.
4. Malamy J, Carr JP, Klessig DF, Raskin I (1990) Salicylic acid: A likely endogenous signal in the resistance response of tobacco to viral infection. *Science* 250:1002–1004.
5. Métraux JP, et al. (1990) Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. *Science* 250:1004–1006.
6. Rasmussen JB, Hammerschmidt R, Zook MN (1991) Systemic induction of salicylic acid accumulation in cucumber after inoculation with *Pseudomonas syringae* pv. syringae. *Plant Physiol* 97:1342–1347.
7. White RF (1979) Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. *Virology* 99:410–412.
8. Ward ER, et al. (1991) Coordinate gene activity in response to agents that induce systemic acquired resistance. *Plant Cell* 3:1085–1094.
9. Görlach J, et al. (1996) Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. *Plant Cell* 8: 629–643.
10. Gaffney T, et al. (1993) Requirement of salicylic acid for the induction of systemic acquired resistance. *Science* 261:754–756.
11. Dong X (2004) NPR1, all things considered. *Curr Opin Plant Biol* 7:547–552.
12. Zhang Y, Tessaro MJ, Lassner M, Li X (2003) Knockout analysis of *Arabidopsis* transcription factors TGA2, TGA5, and TGA6 reveals their redundant and essential roles in systemic acquired resistance. *Plant Cell* 15:2647–2653.
13. Mou Z, Fan W, Dong X (2003) Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. *Cell* 113:935–944.
14. Nawrath C, Métraux JP (1999) Salicylic acid induction-deficient mutants of *Arabidopsis* express PR-2 and PR-5 and accumulate high levels of camalexin after pathogen inoculation. *Plant Cell* 11:1393–1404.
15. Wildermuth MC, Dewdney J, Wu G, Ausubel FM (2001) Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature* 414:562–565.
16. Chen H, et al. (2009) ETHYLENE INSENSITIVE3 and ETHYLENE INSENSITIVE3-LIKE1 repress SALICYLIC ACID INDUCTION DEFICIENT2 expression to negatively regulate plant innate immunity in *Arabidopsis*. *Plant Cell* 21:2527–2540.
17. Gao M, et al. (2009) Regulation of cell death and innate immunity by two receptor-like kinases in *Arabidopsis*. *Cell Host Microbe* 6:34–44.
18. Reddy VS, Ali GS, Reddy AS (2002) Genes encoding calmodulin-binding proteins in the *Arabidopsis* genome. *J Biol Chem* 277:9840–9852.
19. Wang L, et al. (2009) *Arabidopsis* CaM-binding protein CBP60g contributes to MAMP-induced SA accumulation and is involved in disease resistance against *Pseudomonas syringae*. *PLoS Pathog* 5:e1000301.
20. Cao H, Bowling SA, Gordon AS, Dong X (1994) Characterization of an *Arabidopsis* mutant that is nonresponsive to inducers of systemic acquired resistance. *Plant Cell* 6: 1583–1592.
21. Glazebrook J, Rogers EE, Ausubel FM (1996) Isolation of *Arabidopsis* mutants with enhanced disease susceptibility by direct screening. *Genetics* 143:973–982.
22. Parker JE, et al. (1996) Characterization of eds1, a mutation in *Arabidopsis* suppressing resistance to *Peronospora parasitica* specified by several different RPP genes. *Plant Cell* 8:2033–2046.
23. Li X, Zhang Y, Clarke JD, Li Y, Dong X (1999) Identification and cloning of a negative regulator of systemic acquired resistance, SNI1, through a screen for suppressors of npr1-1. *Cell* 98:329–339.
24. Lee J, et al. (2007) Analysis of transcription factor HY5 genomic binding sites revealed its hierarchical role in light regulation of development. *Plant Cell* 19:731–749.

How do plants achieve immunity? Defence without specialized immune cells

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Abstract | Vertebrates have evolved a sophisticated adaptive immune system that relies on an almost infinite diversity of antigen receptors that are clonally expressed by specialized immune cells that roam the circulatory system. These immune cells provide vertebrates with extraordinary antigen-specific immune capacity and memory, while minimizing self-reactivity. Plants, however, lack specialized mobile immune cells. Instead, every plant cell is thought to be capable of launching an effective immune response. So how do plants achieve specific, self-tolerant immunity and establish immune memory? Recent developments point towards a multilayered plant innate immune system comprised of self-surveillance, systemic signalling and chromosomal changes that together establish effective immunity.

Phytopathogens
Microbial organisms that are specialized in attacking plant hosts. They use a variety of infection strategies, ranging from feeding on live plant cells to destroying plants cells to feed on their contents.

Callose
Following pathogen infection, this polysaccharide is produced by plant cells and deposited near the site of attempted penetration to reinforce the cell wall.

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An optimal immune system for long-lived organisms requires high specificity, self-tolerance and immune memory. The immune system in jawed vertebrates is the best studied and most sophisticated. Here, the relatively nonspecific innate immune system is complemented by the highly refined adaptive immune system, which uses vast repertoires of structurally similar receptors — namely, B cell immunoglobulins and T cell receptors (TCRs) — that have an almost infinite number of antigen-binding specificities generated through somatic recombination and mutation. These receptors are clonally expressed by lymphocytes (B and T cells), which travel through the circulatory system to detect pathogens or mutated cells. Antigen recognition by a receptor leads not only to the clonal expansion of lymphocytes expressing that receptor, but also to the formation of long-lived memory cells that produce receptors with the same antigen-binding specificity, allowing secondary immune responses to the corresponding antigen to be faster and more effective.

By comparison, the immune system of plants seems to be far less complex. Because plants lack a circulatory system and mobile immune cells, they cannot use circulating immune receptors to detect non-self. Nonetheless, plants are capable of establishing immune responses that are highly specific, with restricted self-reactivity, and that often generate a lifelong 'memory' of the encountered pathogens. So, these features of vertebrate immunity can be achieved in plants using different immune strategies. The intriguing question is: how does the plant immune system do this?

The initial obstacle that phytopathogens encounter is the plant cell wall, which can be reinforced by the deposition of callose (glucan polymers) following the activation of host defence pathways. The first active line of defence occurs at the plant cell surface when microorganism-associated molecular patterns (MAMPs) — such as lipopolysaccharides, peptidoglycans and bacterial flagellin — are detected by pattern-recognition receptors (PRRs) (BOX 1). Although there are some overall structural similarities between PRRs from plants and animals, such as the use of the leucine-rich repeat (LRR) domain for ligand binding[1], they are thought to have arisen through convergent evolution rather than divergent evolution[1–3]. This is exemplified by the analogous transmembrane flagellin receptors FLAGELLIN-SENSITIVE 2 (FLS2) in *Arabidopsis thaliana* and Toll-like receptor 5 (TLR5) in humans, which are only similar in terms of the LRR domain. Although both receptors recognize conserved epitopes of bacterial flagellin, they bind to different epitopes within this MAMP[4,5]. Moreover, a recent finding indicates that FLS2 might have a different substrate range than TLR5, as it can also detect additional, structurally unrelated MAMPs[6]. These differences in the structures of the PRRs, as well as in their downstream signalling components, indicate that pattern-triggered immunity arose independently in plants and animals.

To circumvent pattern-triggered immunity, adapted pathogens can deliver effector molecules directly into the plant cell. For example, *Pseudomonas syringae* strains

Box 1 | **Plant immune responses induced by pattern-recognition receptors**

Pattern-recognition receptors (PRRs) are found in both plants and animals, and they enable the detection of microorganism-associated molecular patterns (MAMPs). In plants, PRRs are membrane-bound receptor-like kinases (RLKs) or receptor-like proteins (RLPs)[92]. The *Arabidopsis thaliana* genome encodes a large number of RLKs (~615); this property is reminiscent of the sea urchin genome, which unlike that of other animal species contains a recently expanded repertoire of transmembrane receptors (comprising some 222 Toll-like receptor genes)[93]. Some of the *A. thaliana* RLKs are involved in immunity, whereas others have key roles in plant development, symbiosis and self-incompatibility in pollination. XA21 in rice (*Oryza sativa*) was the first PRR to be identified, and it confers resistance against diverse strains of the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae*. However, the ligand for XA21 was cloned only recently and found to be a sulphated peptide of the protein Ax21 (activator of XA21-mediated immunity), which is present in all sequenced *Xanthomonas* species and is predicted to function as an inducer of quorum sensing[94–96]. MAMP–PRR interaction in plants was first studied between the amino-terminal 22 amino acids of flagellin (flg22) of *Pseudomonas syringae* and the flg22 receptor, FLAGELLIN-SENSITIVE 2 (FLS2), in *A. thaliana*[97]. FLS2 contains an extracellular leucine-rich repeat (LRR) domain for ligand binding, a transmembrane domain and a serine/threonine kinase domain. Notably, some plant PRRs (such as the chitin receptor CEBIP in rice) contain a lysine motif rather than the LRR domain for ligand recognition. FLS2 is localized in the plasma membrane and is endocytosed following binding to flg22 (REF. 98). When activated, FLS2 interacts with the co-receptor BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) to initiate pattern-triggered immunity[99,100]. The signal-specific activation of plant PRRs by different MAMPs leads to seemingly generic responses. These responses include ion fluxes, the oxidative burst, activation of a downstream mitogen-activated protein kinase cascade, transcriptional changes and the production of antimicrobial compounds, such as pathogenesis-related (PR) proteins (BOX 2) and phytoalexins.

Convergent evolution
A process by which organisms from different lineages independently evolve similar traits that help them to adapt to their environment.

Pattern-triggered immunity
A basal type of immunity conferred by the recognition of conserved microorganism-associated molecular patterns by specific transmembrane receptors that protect hosts against non-specialized pathogens.

Effector molecules
Pathogen-produced proteins that are injected into the host cell, where they suppress the function of host immune regulators to promote pathogen virulence.

Effector-triggered immunity
A type of immunity triggered by resistance (R) proteins that sense perturbations of host signalling hubs caused by pathogen-produced effector molecules. Effector-triggered immunity frequently culminates in programmed cell death of the infected cell.

Hypersensitive response
A plant immune response that occurs locally to isolate and prevent the growth of pathogens or insects whose life cycles depend on live host cells. This response is triggered when the presence of a pathogen effector is detected by a host resistance (R) protein and is characterized by the rapid death of cells at the infection site.

Programmed cell death
Unlike cell senescence, this is an active form of cell death that occurs through a regulated process during normal development and has a physiological function.

Systemic acquired resistance
A long-lasting, broad-spectrum immune response that is induced throughout the entire plant following attempted local infection.

contain dozens of such effectors. Some of these effectors, such as AvrPto1 of *P. syringae* pv. *tomato*, have been shown to promote pathogen virulence by suppressing immune-related proteins[7,8]. Through co-evolution with pathogens, plants have developed intracellular immune receptors known as resistance (R) proteins that can recognize the presence of certain pathogen effector molecules. Thus, plants can use these immune receptors to detect pathogen 'avirulence' signals and activate effector-triggered immunity. The hallmark of effector-triggered immunity (in other words, R gene-mediated resistance) is a hypersensitive response. This response is typically associated with programmed cell death of the infected cells and the production of antimicrobial molecules — such as the hydrolytic enzymes chitinase and β-1,3-glucanase — in the surrounding tissue, leading to local resistance to the pathogen. Unlike pattern-triggered immunity, which is a general response to a limited number of MAMPs that are conserved between the major microbial groups (for example, fungi and Gram-positive and Gram-negative bacteria), effector-triggered immunity is specific for effectors that are highly polymorphic between different pathogen strains. With a given genome size, how do plants recognize the virtually unlimited number of pathogen effectors?

A local hypersensitive response can also 'immunize' plants against future infection. This phenomenon was named systemic acquired resistance (SAR) by A. Frank Ross, who discovered that the local inoculation of tobacco plants with tobacco mosaic virus (TMV) could protect them against infection with not only TMV but also other pathogens[9]. This broad-spectrum resistance could last for at least 20 days in tobacco plants. So what is the systemic signal for SAR? And how do plants 'remember' prior pathogen exposure?

In this Review, we focus on discussing the design principles of effector-triggered immunity that allow plants to respond to a large array of pathogen effectors while avoiding autoimmunity. We also discuss how SAR is established, leading to long-lasting, broad-spectrum immunity throughout the induced plant and possibly in its progeny. This is not intended to be a comprehensive review of all potential mechanisms of plant immunity but rather a discussion that highlights the similarities and differences with mammalian immune systems based on the most recent publications.

Specificity and self-tolerance

Functional genomic surveys of pathogen effectors indicate that these proteins are highly diverse in sequence as well as in molecular function[10–13]. Surprisingly, the cognate R proteins in plants are structurally conserved. Numerous R proteins have been identified (150 in *A. thaliana*[14] and more than 600 in rice (*Oryza sativa*)[15]), and they typically consist of a variable amino terminus followed by a nucleotide-binding site (NBS) domain in the middle and an LRR domain at the carboxyl terminus. Interestingly, these NBS–LRR proteins have a similar domain structure to animal NLR proteins (nucleotide-binding oligomerization domain (NOD)- and LRR-containing proteins), which are intracellular immune receptors. Based on homology modelling to the well-studied potato R protein Rx, a general mechanistic model for R protein activation has been proposed[16]. In the absence of ligand, intramolecular interactions occur between the variable N terminus, the NBS or NOD and the LRR domain of an R protein or NLR protein. This limits nucleotide exchange and hydrolysis in the central NBS or NOD, thereby inhibiting the activity of the receptor[16–18]. Following ligand binding, this intramolecular inhibition is thought to be alleviated, resulting in receptor activation, which is associated with nucleotide exchange and hydrolysis. In addition, receptor activation leads to possible conformational changes — mediated by interaction with a conserved eukaryotic chaperone complex that contains heat shock protein 90 (HSP90) and suppressor of G2 allele of SKP1 (SGT1) — and to downstream signalling events[18,19].

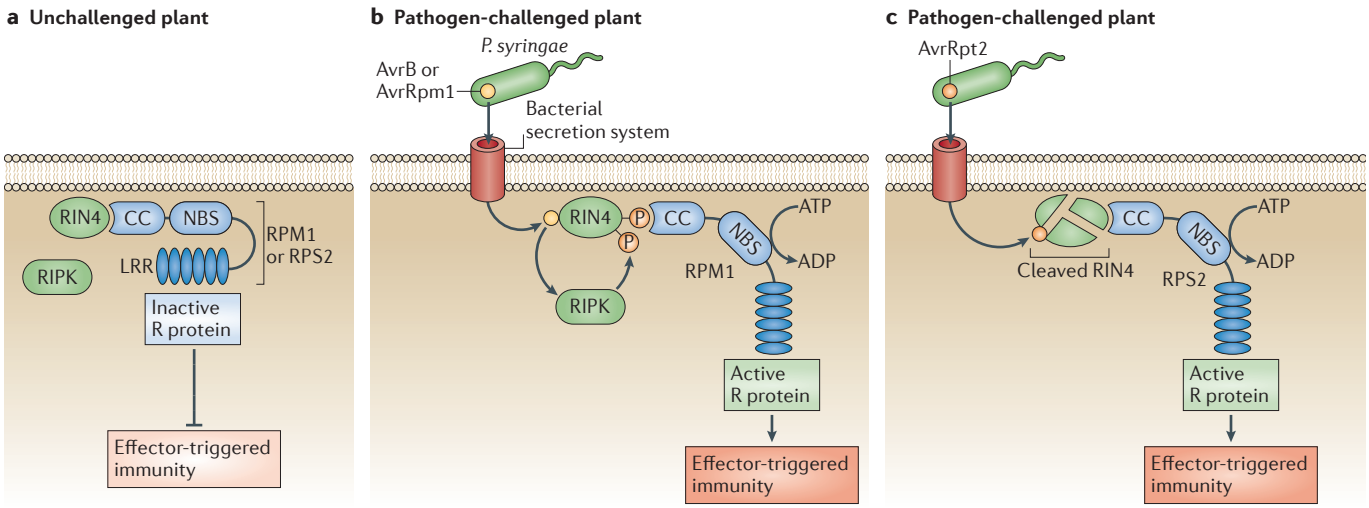

Figure 1 | **The guard model: surveillance of the host immune regulator RIN4 by the R proteins RPM1 and RPS2.** **a** | In unchallenged plants, resistance (R) proteins that have a CC–NBS–LRR (coiled coil–nucleotide-binding site–leucine-rich repeat) domain structure detect unmodified RPM1-INTERACTING PROTEIN 4 (RIN4). This interaction maintains these R proteins (which include RPM1 and RPS2) in an inactive state. **b** | Infection with pathogens such as *Pseudomonas syringae*, which injects the effector molecules AvrB and AvrRpm1 into the plant cell, results in RPM1-INDUCED PROTEIN KINASE (RIPK)-mediated phosphorylation of RIN4. Phosphorylated RIN4 is detected by RPM1, resulting in its activation, which possibly occurs through intramolecular conformational changes (as demonstrated for other R proteins). Active RPM1 induces downstream signalling pathways that lead to effector-triggered immunity. **c** | Infection with pathogens such as *P. syringae* that inject the effector molecule AvrRpt2 into the plant cell results in the cleavage of RIN4, which leads to the activation of RPS2 and effector-triggered immunity.

Recognition of pathogen-induced host perturbations. The highly specific nature of R protein-mediated immunity was discovered more than 50 years ago by studies showing that single dominant Mendelian R loci in flax (*Linum usitatissimum*) varieties could confer resistance to specific strains of a rust fungus[20]. However, based on our current knowledge of the number of R genes in plant genomes, the gene-for-gene model that was proposed at the time[20] — which states that each plant R gene matches with an effector-coding gene in the pathogen — cannot explain the broad immune capacity of plants. A similar puzzle in mammalian immune diversity was reconciled by the discovery of somatic DNA rearrangements of immunoglobulin and TCR gene loci during lymphocyte development. In plants, however, although R genes are present in gene clusters that have higher rates of recombination than the genome average[21], no somatic rearrangement events similar to those in mammals have been observed. Moreover, with only a few exceptions[22–25], most of the R proteins studied so far do not interact with their cognate pathogen effectors directly.

To solve this major puzzle in plant immunity, the guard hypothesis was put forward, stating that unlike immunoglobulin and TCR molecules, which are receptors of non-self signals, plant R proteins bind to and 'guard' pathogen-targeted self proteins. R protein activation is triggered when self proteins are perturbed or modified by pathogen effectors. The best-studied R protein-guarded cellular target is the *A. thaliana* protein RPM1-INTERACTING PROTEIN 4 (RIN4). Consistent with RIN4 being a 'guardee' of R proteins, it not only interacts physically with the R proteins RPM1 and RPS2, but is also targeted and modified by three distinct pathogen effectors from *P. syringae* (namely, AvrRpm1, AvrB and AvrRpt2)[26,27]. Recently, purification of a RIN4-containing complex led to the identification of RPM1-INDUCED PROTEIN KINASE (RIPK), a member of the receptor-like cytoplasmic kinase family. RIPK was shown to phosphorylate RIN4 at several threonine residues in response to the pathogen effectors AvrB and AvrRpm1 (REF. 28). Phosphorylation of threonine 166 of RIN4 was particularly important for R protein activation, as RIN4 mutants with a phosphomimetic amino acid at this position could trigger RPM1-mediated immunity even in the absence of the pathogen effectors AvrRpm1 and AvrB[28,29]. By contrast, the activity of AvrRpt2 on RIN4 is more direct, as RIN4 is cleaved by this bacterial cysteine protease, resulting in RPS2 activation[27,30,31] (FIG. 1).

These findings lead to the next question: why is RIN4 a favourable target for pathogen effectors? To answer this question, the normal cellular function of RIN4 has to be investigated. Studies carried out in plants that do not express the RIN4-guarding R proteins RPM1 and RPS2 show that the *P. syringae* effectors AvrRpm1, AvrB and AvrRpt2 target RIN4 to suppress pattern-triggered immunity, indicating that RIN4 has an important role in plant host defence[26–29,31,32]. However, the molecular mechanism by which RIN4 regulates host defence is largely unknown. One possible mechanism was hinted at by the discovery that RIN4 interacts with plasma membrane-associated H^+-ATPases that regulate the apertures of plant stomata, which are a primary site of pathogen entry into the plant leaf and a major target of MAMP action[33].

NLR proteins
(Nucleotide-binding oligomerization domain (NOD)- and leucine-rich repeat (LRR)-containing proteins). A group of intracellular immune receptors that have a structure that closely resembles that of resistance (R) proteins in plants. In contrast to R proteins, NLRs in mammals detect microorganism-associated molecular patterns rather than pathogen effectors.

Taken together, these data strongly suggest that the plant immune system uses R proteins predominantly to monitor pathogen effector-triggered perturbations of self molecules, rather than to detect non-self molecules (FIG. 1). This strategy provides plants with the potential to specifically recognize groups of pathogens that use similar infection strategies (in other words, pathogens that use effectors converging on the same host targets, such as RIN4). Despite the numerous different effectors that pathogens inject into plant cells to promote virulence, they might target relatively few conserved 'hubs' in the plant signalling network that controls plant defence, metabolism and signalling[34]. These signalling hubs are probably essential host proteins and so might be difficult to identify through genetic approaches. A recent study used a genome-wide yeast two-hybrid screen to identify physical interactions between *A. thaliana* immune-related proteins (including 30 R proteins) and effectors from two evolutionarily separated pathogens. In this study, it was shown that pathogen effectors might converge on a limited set of host proteins that are highly interconnected hubs with important regulatory roles in plant immune signalling[35]. Moreover, rather than physically associating with pathogen effectors, plant R proteins were found to interact with effector-targeted host proteins. This work provided crucial information regarding how a few hundred R genes might be sufficient to protect plants from a much larger array of potential pathogen-encoded effectors.

R protein-mediated programmed cell death. The (indirect) activation of R proteins by pathogen effectors often leads to programmed cell death at the site of attempted infection. This defence strategy is effective against viral, bacterial, fungal and oomycete pathogens, as well as nematodes that feed on live plant cells. However, unlike NLRs in animals, which are known to trigger cell apoptosis through the activity of caspases that activate pro-inflammatory cytokines[36], plants lack the homologous caspases, and several potential alternative mechanisms have been proposed for R protein-mediated programmed cell death. A recent publication shows that one type of β-subunit of the 26S proteasome (namely, PBA1) contributes to the caspase-3-like activity that is observed during the resistance response in *A. thaliana* to the bacterial pathogen *P. syringae* pv. *tomato*[37]. This activity is required for membrane fusion between the central vacuole and the plasma membrane of the plant cell, which leads to the release of antibacterial factors and programmed cell death-promoting signals from the vacuole, and consequently pathogen resistance. Another recent paper indicates that R protein-triggered programmed cell death can be mediated by metacaspases. Specifically, *A. thaliana* METACASPASE 1 (AtMC1) was shown to function as a positive regulator of programmed cell death. Elimination of its catalytic residues rendered AtMC1 unable to trigger cell death[38]. The notion that R protein-mediated programmed cell death might involve perturbation of multiple cellular processes came from a report showing that, in *A. thaliana*, the resistance conferred by the R protein RPP4 against the obligate biotroph *Hyaloperonospora arabidopsidis* is not mediated by a single gene but rather by multiple downstream genes[39]. Phenotypic analysis of plants with mutations in these genes showed that programmed cell death is the major defence mechanism against *H. arabidopsidis*. Interestingly, these programmed cell death-promoting genes encode proteins, mostly enzymes, with very diverse functions, including a receptor-like kinase, a calcineurin-like phosphoesterase, a protease, a UDP-glucosyl transferase, an ABC transporter and an ATPase. However, it is still debatable whether cell death is the cause or a consequence of resistance, because in some mutant plants as well as in transgenic plant cell lines that express cell death inhibitors, cell death is abolished but R protein-mediated pathogen resistance is not perturbed[38,40,41]. It is plausible that, on these genetic backgrounds, pathogen growth is blocked before the R protein-mediated induction of cell death. Epistasis experiments may be helpful for testing this hypothesis.

Strategies to prevent autoimmunity. As R proteins are expressed by all plant cells, controlling their activity is crucial for plant survival, as well as for defence against pathogens. Plant R proteins have evolved to recognize modified self antigens, so there should be strong selective pressure to eliminate R proteins that can be activated by normal (unmodified) self antigens. Nonetheless, recent findings indicate that this design principle of the plant immune system can occasionally give rise to autoimmunity in genetically diverse populations. For example, in *A. thaliana* (a predominantly self-pollinating species), about two percent of manually performed intraspecific crosses result in offspring that are severely necrotic, sterile or nonviable[42]. This phenomenon, known as hybrid necrosis, is associated with the spontaneous, systematic activation of immune-related genes. Mapping of the loci responsible for hybrid necrosis has repeatedly identified R genes[42–44], indicating that the inherent self-tolerance of R protein-mediated immunity might be compromised by incompatible genetic interactions. Hybrid necrosis occurs when R genes from one parent plant are mixed with a corresponding incompatible target locus (potentially encoding an R protein guardee) from the other parent plant. An important clue to the nature of such a locus was recently revealed in a study of hybrid necrosis caused by interspecific crosses in lettuce (*Lactuca sativa*). One of the two interacting loci found in this study encoded a RIN4 orthologue. Interestingly, substitution of three polymorphic residues of RIN4 in one parent with the corresponding residues of the other parent averted necrosis in the hybrid offspring[45]. In another study, it was shown that autoimmunity arose from incompatible interactions between the *A. thaliana RPP1* cluster of R genes and allelic variations in the gene encoding the receptor-like kinase STRUBBELIG-RECEPTOR FAMILY 3 (SRF3), indicating that *RPP1*-cluster proteins might monitor SRF3 for perturbations induced by pathogen effectors[46]. Taken together, these findings imply that intraspecific or interspecific crosses can lead to a mismatch between R proteins and the targets of pathogen effectors that they

Metacaspases
Arginine- and lysine-specific proteases that are related to animal caspases. Metacaspases are found in plants, fungi and protists, where they have an essential role in programmed cell death responses.

Hybrid necrosis
A post-zygotic incompatibility resulting from intraspecific or interspecific crosses that is typified by severe tissue necrosis, stunting and auto-activation of immune responses.

guard. Consequently, in hybrid plants, R proteins from one parent recognize the effector targets from another parent as modified self, and this results in auto-activation of the R protein in the absence of pathogen attack (FIG. 2). As noted by others[44], it will be interesting to determine how autoimmunity is avoided in outcrossing plants, such as maize, in which hybrid necrosis has not been observed despite the intensive mixing of heterologous genetic backgrounds during domestication.

Another important research focus for understanding the mechanism that controls autoimmunity is how R protein signalling is normally turned off in plants. Gain-of-function mutations in the R genes *SNC1* and *SSI4* in *A. thaliana* indicate that dysregulation of R proteins poses an imminent autoimmune threat. Mutant *snc1* and *ssi4* plants show signs of autoimmunity — including immune-related transcriptional reprogramming, accumulation of hydrolytic enzymes with antimicrobial activities, and spontaneous cell death that resembles that induced by the hypersensitive response — and this culminates in stunted growth and altered morphology[47,48]. It is therefore likely that the activities of R proteins are normally under strict cellular control. Several reports have shown that the overexpression of R proteins results in autoimmunity[49,50], indicating that the activity levels of some R proteins are linked to their cellular levels. Indeed, it was recently shown that mutation of the gene encoding the tetratricopeptide repeat domain-containing protein SRFR1 resulted in autoimmune responses owing to transcriptional upregulation of several co-regulated R genes[51,52]. Accordingly, SRFR1 has substantial sequence similarity to various eukaryotic transcriptional repressors in which the tetratricopeptide repeat interacts with other transcriptional (co)regulators[53]. In another recent study, protein degradation mediated by a SKP1–CULLIN 1–F-box protein complex was shown to have a role in controlling R protein levels, as a loss-of-function mutation in the gene encoding the F-box protein CPR1 resulted in the accumulation of higher levels of the R proteins SNC1 and RPS2, as well as in autoimmunity[54]. The autoimmune phenotype of the *cpr1* mutant was largely suppressed by knocking out *SNC1*, indicating that it was the result of R protein over-accumulation. It is plausible that high levels of R proteins out-titrate regulatory factors, such as chaperone complexes, that normally control their activities. R protein stability is tightly controlled by a highly conserved eukaryotic chaperone complex that includes HSP90, SGT1 and the cysteine- and histidine-rich domain-containing protein RAR1. It is thought that this chaperone complex maintains R proteins in a recognition-competent state and, after they recognize pathogen effector-modified self proteins, facilitates the conformational change of R proteins to induce downstream immune signalling. Accordingly, mutation of genes encoding chaperone components markedly affects R protein stability[19].

R protein levels, and thus their activities, are not simply under constitutive or static cellular control. Rather, they can follow a dynamic pattern of expression and accumulation. The *A. thaliana* R gene *RPP4*, which confers immunity against the downy mildew disease agent *H. arabidopsidis*, was shown to adopt a rhythmic pattern of expression controlled by the circadian regulator CCA1. Intriguingly, peak expression of *RPP4* and RPP4-dependent genes occurred at dawn, coinciding with the time of *H. arabidopsidis* sporulation[39]. Accordingly, artificial infection with *H. arabidopsidis* at dusk increased the susceptibility of plants to this pathogen as compared with infection at dawn. Thus, plants seem to anticipate infection by *H. arabidopsidis* strains at dawn through the regulated expression of particular R genes. It is plausible that this mechanism is widely used by plants to minimize the risk of autoimmunity, as these R genes are only temporarily expressed when necessary.

In summary, signal-specific immunity in plants is provided by structurally similar R proteins that guard key cellular signalling hubs. Perturbation of these hubs by pathogen effectors activates R proteins, which trigger a programmed cell death response and establish immunity. The intrinsic autoimmune reactivity of R proteins requires plant cells to tightly regulate their expression and activity to minimize self-reactivity.

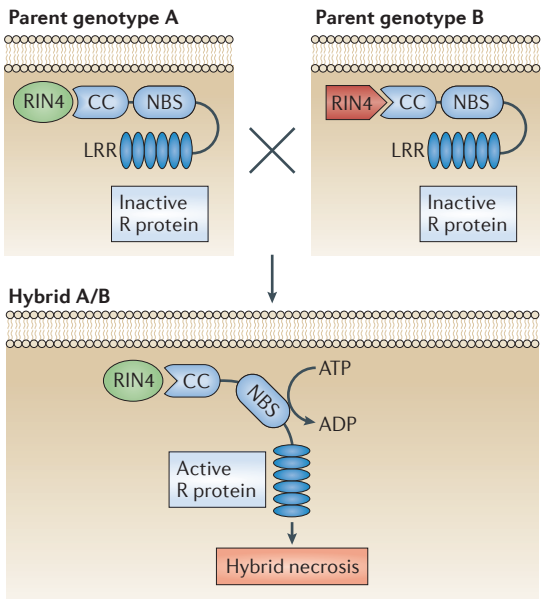

Figure 2 | **Autoimmunity in necrotic hybrids might be caused by a mismatching of R proteins and the targets of pathogen effectors that they guard.** The figure shows a proposed model for the involvement of resistance (R) proteins and the host targets of pathogen effectors in hybrid necrosis. Parental genotypes A and B have sequence variations in the effector target RPM1-INTERACTING PROTEIN 4 (RIN4) and the R proteins that guard RIN4. Hybrid progeny inherit RIN4 variants and the respective guarding R proteins from both parents. Consequently, the R protein from one parent might recognize RIN4 from the other parent as a modified protein, resulting in the activation of immune responses in the absence of pathogen challenge and causing autoimmune-induced hybrid necrosis. CC, coiled coil; LRR, leucine-rich repeat; NBS, nucleotide-binding site.

Systemic activation of immunity

Mobile immune cells and secreted opsonins (antibodies) of the humoral immune system provide animals with specific immunity throughout their entire body. Plants do not have a circulatory system, but the experiments outlined below indicate that their non-circulatory vascular system nonetheless transports immune signals from the site of infection to systemic uninfected tissues to establish SAR. Unlike adaptive immunity in animals, which is antigen specific, SAR is typically induced following effector-triggered immunity (although induction by pattern-triggered immunity has also been reported[55]) and is effective against a wide range of biotrophic pathogens[56].

Mobile immune signals. Since the discovery of SAR, much research has been devoted to identifying the mobile immune signal that is responsible for this phenomenon. Such a signal should be generated in the infected tissue and be rapidly transported to uninfected parts of the plant. It might also be able to encode detailed information about the primary pathogen infection. If the last criterion holds true, then more than one kind of mobile signal, functioning in a synergistic manner, might be needed to relay such complex information to systemic tissues. The currently available data seem to support this hypothesis.

The onset of SAR is accompanied by increased accumulation of the signalling hormone salicylic acid in the phloem[57]. Moreover, the removal of salicylic acid by constitutive expression of a salicylate hydroxylase abolishes SAR[58]. Although these findings strongly suggest that salicylic acid is a transported immune signal, grafting experiments showed that salicylic acid is dispensable for signal generation at the site of infection; rather, it is required for SAR development in systemic tissues[59]. More-recent grafting experiments using tobacco plants showed that salicylic acid methyltransferase activity, which converts salicylic acid into methylsalicylic acid (MeSA), is required in the tissue that generates the immune signal. Conversely, MeSA esterase activity, which converts MeSA back into salicylic acid, is required for signal perception in systemic tissues. Taken together with the observation that MeSA accumulates in the phloem following the activation of SAR, these results suggest that MeSA might be the transported immune signal[60]. Although exogenous application of MeSA induced systemic immunity in wild-type tobacco plants[60], it is not known whether MeSA can bypass the requirement for salicylic acid methyltransferase to induce SAR. Moreover, experiments using *A. thaliana* found that knockout mutant plants that lacked salicylic acid methyltransferase failed to accumulate MeSA, but still retained the ability to systemically accumulate salicylic acid and activate SAR. In fact, most of the MeSA produced following infection of *A. thaliana* escaped the plant by volatile emission[61]. These data indicate that, contrary to the findings made in tobacco plants, MeSA is dispensable for SAR in *A. thaliana*. Thus, it remains uncertain whether MeSA is a necessary and/or sufficient mobile immune signal for SAR in general. It seems that the composition of the mobile immune signal in SAR might differ depending on the plant species and the type of plant–pathogen interaction.

A labour-intensive genetic screen for mutant *A. thaliana* plants deficient in systemic immunity identified the *defective in induced resistance 1-1* (*dir1-1*) gene[62]. Importantly, mutant *dir1-1* plants can still launch local immune responses, indicating that *DIR1* is only required for systemic immunity. Vascular exudates from pathogen-inoculated wild-type plants induced immune-related genes, whereas those from mutant *dir1-1* plants did not. In accordance with this, and the fact that *DIR1* is predicted to encode an apoplastic lipid-transfer protein, it was concluded that DIR1 has a role in immune signal generation and/or transports a lipid-based immune signal to systemic tissues. The hormone jasmonic acid fits the profile for such an immune signal, as it is a lipid-derived molecule and its accumulation in the phloem is associated with the induction of SAR[63]. However, mutant plants with defects in jasmonic acid biosynthesis or signalling have varying degrees of SAR. This was again dependent on the type of plant–pathogen interaction[61,63], thereby casting doubts on the role of jasmonic acid as a mobile immune signal. Indeed, co-infiltration of jasmonic acid or methyl jasmonate with vascular exudates from SAR-deficient plants failed to induce pathogen resistance. Moreover, fractionation of SAR-induced vascular exudates revealed that jasmonic acid did not co-purify with the SAR-inducing activity[64].

A breakthrough was made with the discovery that *A. thaliana* mutants with an impairment in the biosynthesis of the organophosphate compound glycerol-3-phosphate (G3P) failed to activate SAR[65,66]. Importantly, the development of SAR in distal tissues was rescued in these mutant plants by the local application of exogenous G3P or SAR-induced vascular exudates from wild-type plants. Conversely, SAR-induced vascular exudates from mutant plants with defective G3P biosynthesis failed to induce SAR in wild-type plants unless supplemented by G3P[66]. These data imply that G3P is a signal that is generated following the infection of primary tissues and subsequently translocated to distal parts of the plant to induce systemic immunity. Intriguingly, the authors reported that exogenous G3P was most effective in inducing SAR when it was applied together with vascular exudates from mock-treated plants, which indicates that a cofactor might be required for the immune activity of G3P. Indeed, G3P-induced SAR was shown to be dependent on DIR1 and vice versa. Although a physical association between G3P and DIR1 was not found, these findings strongly suggest that cooperative movement of these mobile immune signals confers SAR.

In addition to G3P, azelaic acid has been identified as a mobile immune signal through the analysis of infection-induced plant vascular exudates[67]. Azelaic acid was shown to prime plants for salicylic acid accumulation and the activation of immune-related genes. Moreover, it induced the expression of *AZELAIC ACID INDUCED 1* (*AZI1*), which is predicted to encode a secreted lipid-transfer protein. Reciprocal application of vascular exudates from wild-type and *azi1* mutant plants indicated that AZI1 is involved in the production and/or translocation of a mobile immune signal.

Phloem
The plant vascular tissue, which transports organic nutrients (such as sugars) from photosynthetic 'source' tissues to nutrient-consuming 'sink' tissues throughout the entire plant.

Apoplastic
Localized to the free diffusional space outside the plasma membrane of plant cells.

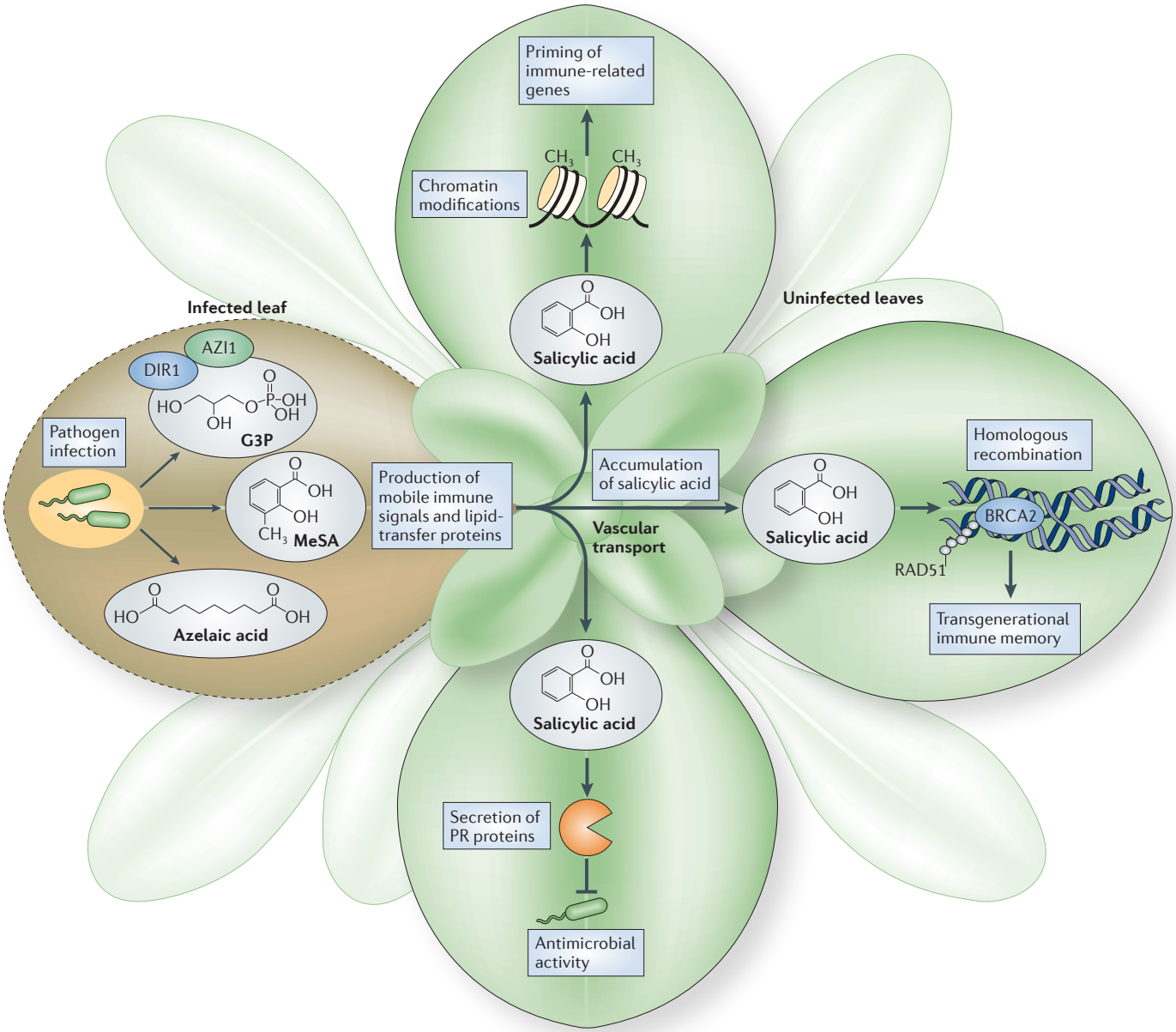

Figure 3 | **Translocation of mobile immune signals induces systemic immunity and immune memory.** Local pathogen infection results in the production of the mobile immune signals methylsalicylic acid (MeSA), azelaic acid and glycerol-3-phosphate (G3P), and the lipid-transfer proteins DEFECTIVE IN INDUCED RESISTANCE 1 (DIR1) and AZELAIC ACID INDUCED 1 (AZI1). These mobile signals are transported through the vasculature to systemic, uninfected parts of the plant, where through an unknown mechanism they induce the accumulation of salicylic acid, which is a signal molecule for systemic acquired resistance. Accumulation of salicylic acid induces: the secretion of pathogenesis-related (PR) proteins with antimicrobial activities; histone methylation and other chromatin modifications that prime immune-related genes for increased expression and establish immune memory; and somatic homologous recombination through the actions of BREAST CANCER SUSCEPTIBILITY 2 (BRCA2) and RAD51 to potentially establish a transgenerational memory of immunity.

Taken together, the data indicate that the mobile immune signal in plants consists of multiple proteins as well as lipid-derived and hormone-like molecules (FIG. 3). There are several indications that these different components coordinate each other's activities to establish systemic immunity. For example, azelaic acid could not induce immunity in the *dir1-1* mutant plants, indicating that its activity requires DIR1 (REF. 67). Moreover, *dir1-1* mutant or *DIR1*-silenced plants have increased expression of salicylic acid methyltransferases, resulting in increased production of MeSA at the cost of salicylic acid accumulation and disease resistance[68]. It is plausible that the interplay between different mobile immune signals in plants might relay specific information about the type of primary pathogen encountered and consequently determine the level of immune response that is most appropriate for systemic tissues.

Cellular reprogramming prioritizes immunity. Early studies showed that the arrival of mobile immune signals in systemic tissues is associated with an increased accumulation of salicylic acid[57–59]. However, the mechanism by which this is accomplished is largely unknown, except for the conversion of mobile MeSA to salicylic acid as described above. The regulation of salicylic acid metabolism in plants by the salicylic acid biosynthetic enzyme isochorismate synthase and the salicylic acid-inactivating enzyme salicylic acid glucosyltransferase might hold the key to this question and should be studied in more detail.

Signalling downstream of salicylic acid has been studied intensely because the exogenous application of salicylic acid to plants can mimic pathogen-induced SAR. This method, which was serendipitously discovered using aspirin (acetylsalicylic acid)[69], is not only convenient for conducting genetic screens, but also the basis for the development of synthetic salicylic acid analogues — such as 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) — for commercial use in controlling plant disease. Treating *A. thaliana* plants with these compounds results in marked transcriptional changes in more than 2,000 genes, including those that encode pathogenesis-related (PR) proteins with antimicrobial activity[70] (BOX 2). This transcriptional reprogramming is largely dependent on the transcription cofactor NONEXPRESSER OF PR GENES 1 (NPR1) (BOX 3). NPR1 not only activates a myriad of immune-related genes (such as PR genes) and genes encoding transcription factors that initiate further transcriptional cascades, it also downregulates genes involved in basic cellular processes, such as photosynthesis, thereby prioritizing immune responses at the cost of plant growth[70,71].

Box 2 | **Pathogenesis-related proteins are the executioners of plant immunity**

The production of pathogenesis-related (PR) proteins was first associated with tobacco mosaic virus (TMV) infection of tobacco plants[101]. Later studies showed that PR proteins include hydrolytic enzymes (such as β-1,3-glucanase and chitinase) and defensins, which have potent antimicrobial activities through the hydrolysis of pathogen cell walls and the disruption of the pathogen membrane, respectively. Their synthesis is induced not only by pathogens, but also by immune signals such as salicylic acid in the absence of pathogen challenge. Therefore, the term 'PR proteins' is really a misnomer, as these antimicrobial proteins are the executioners of plant immunity. Fourteen classes of PR protein (PR1–PR14) are currently recognized in plants[102]. Early efforts in engineering disease resistance in plants through the overexpression of PR proteins showed that they are not as effective when induced individually compared with when they are coordinately expressed[103]. It is known that distinct sets of PR proteins are induced in response to different pathogens. In *Arabidopsis thaliana*, PR1, PR2 (a β-1,3-glucanase) and PR5 (thaumatin) are induced by salicylic acid in response to biotrophic pathogens, whereas PR3 (a chitinase), PR4 (a chitinase) and PR12 (a defensin) are induced by jasmonic acid in defence against necrotrophic pathogens[104]. Moreover, the regulation of a large set of endoplasmic reticulum-resident proteins is required to ensure proper folding, transport and secretion of PR proteins[105]. Plant genomes have the capacity to produce a large array of PR proteins. For defensins alone, 317 defensin-like sequences were found through a search of the *A. thaliana* genome[106]. The defensin genes are present in clusters, probably as a result of gene duplication and divergent or purifying selection. Defensins are found not only in plants, but also in insects and mammals, and they have diverse immune functions against bacterial and fungal pathogens as well as herbivorous insects. Therefore, understanding the regulation and the activities of PR proteins is a crucial part of immunological research.

Taken together, the available data indicate that the establishment of systemic immunity involves the transport of multiple mobile signals from the site of initial infection to the entire plant. The perception of these signals in systemic tissues leads to the accumulation of salicylic acid, which mediates transcriptional reprogramming through activation of the co-activator NPR1. Ultimately, this results in the expression of antimicrobial proteins, the concerted action of which confers broad-spectrum pathogen resistance.

Immune memory

Adaptive immunity in animals confers long-lasting resistance after primary antigen recognition owing to the formation of memory immune cells. Consequently, secondary exposure to the same antigen triggers an accelerated and more-effective immune response. Despite the absence of specific memory immune cells, the SAR response in plants also confers a long-lasting memory of primary pathogen attack but is far less specific than adaptive immune memory in animals. Consequently, SAR provides an enduring, heightened state of resistance against secondary attack by a broad range of pathogens. Moreover, some studies seem to indicate that this immune memory not only can provide life-long protection for the plant, but might also be transmitted to subsequent generations.

Establishing long-lasting immunity. Similarly to investigations of SAR in plants, studies of immunity in invertebrate animals (which also do not have a typical adaptive immune response) show that primary pathogen exposure can induce life-long protection against secondary infection[72], indicating that immune memory might be a common phenomenon. Immune memory in plants and invertebrate animals has been associated with cell priming, which results in a sensitized state that allows cells to respond faster and with greater amplitude to secondary pathogen attack, thereby rapidly limiting pathogen proliferation and spread. However, the molecular mechanisms of cell priming are not well understood. Priming is widely speculated to result from the cellular accumulation of signalling components that are activated only following exposure to a secondary pathogen attack. Recent work in plants seems to confirm this hypothesis. In *A. thaliana*, chemical- or pathogen-induced priming correlates with the accumulation of the mitogen-activated protein kinases MPK3 and MPK6 (at both the mRNA transcript and protein levels). However, these kinases are maintained in an inactive state in primed cells and require pathogen or chemical challenge for activation[73]. Interestingly, priming of MPK3 and MPK6 expression was abolished in *npr1* mutant plants. This is consistent with the previously mentioned role of NPR1 in the transcriptional reprogramming of cells to prioritize immunity. Moreover, primary pathogen attack was previously shown to induce the accumulation of transcriptionally active NPR1 monomers in systemic tissues[74]. Thus, the accumulation of immune signalling components, such as MPK3, MPK6 and NPR1, could confer long-lasting resistance to secondary pathogen attack.

Box 3 | **Regulation and function of the systemic acquired resistance co-activator NPR1**

Several reports have shown that salicylic acid and its analogues trigger transient oxidative and reductive changes in plant cellular redox states[107]. These changes regulate the conformation of NONEXPRESSER OF PR GENES 1 (NPR1), allowing it to switch reversibly between a disulphide bond-mediated oligomeric complex and a monomeric state in the cytoplasm[74,108]. Monomeric NPR1 translocates to the nucleus, where it forms a complex with members of the TGA family of transcription factors, some of which may also undergo redox-regulated conformational changes[109,110]. The marked change in global transcription induced by NPR1 is reminiscent of that induced by the master immune regulator nuclear factor-κB (NF-κB) in mammals. In contrast to the nuclear translocation of NPR1, which is controlled by a redox-sensitive oligomer–monomer exchange, the nuclear translocation of NF-κB occurs when its inhibitor (IκB) is phosphorylated and degraded by the proteasome. In addition to the nucleocytoplasmic regulation of their activities, NPR1 and NF-κB also have in common a pulsatile accumulation in the nucleus (with a period of ~100 minutes for NF-κB; NPR1 pulses have not yet been measured at the single-cell level). For NF-κB, this is largely due to a delayed negative feedback loop created by NF-κB-dependent transcriptional activation of the gene encoding IκB[111–113]. Interestingly, the persistence, period and amplitude of NF-κB pulses seem to differentially activate immune-related genes[112,113]. In the case of NPR1, its transient accumulation in the nucleus as a transcriptionally active monomer is regulated by changes in the cellular redox state, coupled with its proteasome-mediated clearance from the nucleus[108,114,115]. Although the details of how NPR1 pulses control downstream transcriptional events require further investigation, blocking the proteasome-mediated degradation of NPR1 in the nucleus delays and decreases the transcription of certain target genes[114]. This implies that NPR1 proteins that have initiated a transcription event might need to be cleared from the gene promoter to efficiently release RNA polymerase II and/or to reset the promoter to allow the re-initiation of transcription.

It is not completely clear how NPR1 brings about the chromosomal changes that prime target genes for enhanced transcription. Surprisingly, salicylic acid induces the recruitment of RAD51, BREAST CANCER SUSCEPTIBILITY 2 (BRCA2) and SUPPRESSOR OF SNI1 2 (SSN2; a homologue of the yeast protein Sws1) to the promoters of NPR1 target genes[75,76]. These are highly conserved proteins in eukaryotes that are involved in DNA repair and homologous recombination. Moreover, salicylic acid and DNA damaging agents (such as bleomycin) have synergistic effects on immune gene induction (S. Yan and X.D., unpublished observations). It is plausible that chromatin remodelling by proteins involved in DNA repair and homologous recombination might underpin gene priming, but the specific mechanism of this priming effect needs to be further elucidated.

Changes in the methylation and acetylation status of DNA and histones have been associated with the activation of immune-related genes in plants[77]. The latest evidence now indicates that epigenetic modifications might also have an important role in providing plants with a long-lasting immune memory. Local pathogen infection was shown to modify the methylation and acetylation status of histones at gene promoters in systemic tissues[78]. In particular, trimethylation of histone H3 lysine 4 (H3K4me3) at certain gene promoters was strongly induced in distal tissues following local pathogen infection, and this modification correlated with the potentiated expression of immune-related genes following challenge. Intriguingly, both H3K4me3 modification and the associated potentiation of immune gene transcription required NPR1, indicating that this co-activator also orchestrates epigenetic transcriptional poising[78]. Hence, a combination of epigenetic control mechanisms and an abundance of signalling components seems to be responsible for the development of long-lasting immune memory in plants (FIG. 3).

Redox state
A term that can be used narrowly to describe the ratio of interconvertible oxidized and reduced forms of a specific redox couple (such as NAD^+–NADH), but that can also be used broadly to describe the cellular redox environment, which is determined by the states of all of the redox couples combined.

Transgenerational memory of immunity. R genes have been found to reside in clusters within plant genomes. It is thought that such clusters are the result of successive rounds of duplication and unequal recombination, enabling diversification of the genes within these clusters and subsequent selection for greater specificity and effectiveness[14,79]. Curiously, epigenetic changes have been suggested to influence the stability of these gene clusters. Hypomethylation in the *A. thaliana bal* variant (which was generated in the *ddm1* (*decreased DNA methylation 1*) background) was shown to be associated with the tandem duplication of a 55-kb region containing six R genes[80]. Moreover, rearrangements in N gene-like loci, which contain R genes that may confer resistance against TMV, correlate well with local DNA hypomethylation in tobacco plants[81]. In addition, following on from pioneering work in maize[82], more-recent reports have indicated that biotic stress increases genome instability[77,81,83,84]. Taken together, these findings make it tempting to speculate that increased pathogen pressure promotes the formation of new R genes by locally changing the epigenetic chromatin landscape to destabilize R gene clusters and allow for gene rearrangements. The rare R gene recombination events that are beneficial under a particular pathogen pressure could then be inherited by the plant progeny (FIG. 3).

The repressor of plant immunity SNI1 might be involved in regulating the chromatin landscape of immune-related genes. A loss-of-function mutation in *SNI1* changes the acetylation and methylation status of the chromatin encompassing the immune marker gene *PR1*, and this change mimicks the pathogen-induced state of this gene[85]. Mutant *sni1* plants also have enhanced levels of somatic homologous recombination[86], suggesting that SNI1 might control recombination rates through chromatin remodelling. Notably, genetic screens for mutations that suppress the constitutive defence phenotype of *sni1* mutant plants have so far exclusively identified genes involved in DNA repair and homologous recombination, such as *BRCA2* and *RAD51*, implying that the mutant *sni1* phenotype is largely

due to an increase in the activity of DNA repair machinery[75,76,86]. A major future challenge remains to determine whether pathogen-induced DNA rearrangements occur at specific genomic sites, including R gene loci.

How could stress-induced somatic homologous recombination lead to a transgenerational memory of stress? Stress-induced somatic homologous recombination in a cell can lead to the formation of a sector within the plant that has an enhanced stress-resistance trait; such sectors have an advantage within the plant, as they are more successful than other sectors. Unlike animals, plants do not have a preset embryonic germ line; instead, plant gametes arise from somatic tissues. Thus, successful somatic sectors that give rise to gametes allow reproduction and preferential transmission of the stress-resistance trait[87]. Indeed, it was previously reported that an ultraviolet B radiation-induced somatic rearrangement of a reporter construct in *A. thaliana* was stably transmitted to subsequent generations, indicating that somatic homologous recombination events can introduce permanent genetic changes in plant populations[88].

Interestingly, it has been reported that the progeny of parental plants that were exposed to a MAMP or pathogen maintain increased levels of somatic homologous recombination in the absence of pathogen stress[81,83]. This indicates that successive plant generations might be heritably poised to cope with environments of high pathogen pressure. Many abiotic stresses also induce homologous recombination, but in only a select few cases does the increased level of somatic homologous recombination in the parent plant persist in unstressed progeny[83,89,90]. This suggests that transgenerational memory is not a general response to environmental stresses, but is instead specific to certain types of stress, such as pathogen attack. The molecular basis for this phenomenon is likely to be epigenetic and subject to dynamic changes. Such a hypothesis can now be tested, as genome-wide high-resolution mapping of DNA methylation has been carried out in wild-type *A. thaliana* as well as in the DNA methyltransferase-null mutant (*ddc*; for the *drm1*, *drm2* and *cmt3* triple mutant)[91]. Similar surveys can be repeated in response to immune induction in treated parental plants and in the untreated progeny.

In summary, long-lasting immune memory might be established by the enhanced accumulation of signalling components and by epigenetic changes that prime gene promoters. Moreover, it has been suggested that plants can establish transgenerational immune memory through epigenetic changes and by increasing the rate of DNA rearrangement to generate new R genes. But whether this is a widespread phenomenon requires further investigation.

Perspectives

We have described how the plant immune system adopts unique strategies that render it highly pathogen specific with intrinsic autoimmune tolerance owing to R protein-mediated cellular surveillance, which enables plants to induce immunity in distal tissues through the long-distance transport of hormones and lipid-derived molecules. These strategies also provide potentially life-long or transgenerational memory of immunity through cellular priming and somatic homologous recombination. However, it should be noted that we have only discussed defence mechanisms against biotrophic pathogens, which rely on live host cells either completely or partly in their life cycle. Immune responses against necrotrophic pathogens (which feed on dead host cells) and herbivorous insects are mechanistically distinct from or even antagonistic to those used against biotrophs. Nevertheless, these responses are also highly specific and in some cases can generate long-lasting memory. We also know very little about the immune mechanisms used by roots and anticipate that they may be quite different from those used in leaves. Similarly to the digestive tracts of animals, roots are constantly associated with microorganisms, most of which are beneficial to plant health. Therefore, immune responses in roots have to be well controlled to distinguish friend from foe. Major challenges remain in understanding the dynamic and spatial regulation of these various immune responses and their interplay with other cellular functions. There is also a need for molecular studies on how plant immune function and memory operate in large populations and in long-lived plants, such as trees. Hence, plant immunity still has many mysteries that remain to be solved.

1. Mizel, S. B., West, A. P. & Hantgan, R. R. Identification of a sequence in human toll-like receptor 5 required for the binding of Gram-negative flagellin. *J. Biol. Chem.* **278**, 23624–23629 (2003).
2. Ausubel, F. M. Are innate immune signaling pathways in plants and animals conserved? *Nature Immunol.* **6**, 973–979 (2005).
3. Ronald, P. C. & Beutler, B. Plant and animal sensors of conserved microbial signatures. *Science* **330**, 1061–1064 (2010).
4. Felix, G., Duran, J. D., Volko, S. & Boller, T. Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *Plant J.* **18**, 265–276 (1999).
5. Smith, K. D. *et al.* Toll-like receptor 5 recognizes a conserved site on flagellin required for protofilament formation and bacterial motility. *Nature Immunol.* **4**, 1247–1253 (2003).
6. Danna, C. H. *et al.* The *Arabidopsis* flagellin receptor FLS2 mediates the perception of *Xanthomonas* Ax21 secreted peptides. *Proc. Natl Acad. Sci. USA* **108**, 9286–9291 (2011).
7. Jones, J. D. G. & Dangl, J. L. The plant immune system. *Nature* **444**, 323–329 (2006).
8. Zipfel, C. & Rathjen, J. P. Plant immunity: AvrPto targets the frontline. *Curr. Biol.* **18**, R218–R220 (2008).
9. Ross, A. F. Systemic acquired resistance induced by localized virus infections in plants. *Virology* **14**, 340–358 (1961).
10. Petnicki-Ocwieja, T. *et al.* Genomewide identification of proteins secreted by the Hrp type III protein secretion system of *Pseudomonas syringae* pv. tomato *DC3000*. *Proc. Natl Acad. Sci. USA* **99**, 7652–7657 (2002).
11. Baxter, L. *et al.* Signatures of adaptation to obligate biotrophy in the *Hyaloperonospora arabidopsidis* genome. *Science* **330**, 1549–1551 (2011).
12. Cabral, A. *et al.* Identification of *Hyaloperonospora arabidopsidis* transcript sequences expressed during infection reveals isolate-specific effectors. *PLoS ONE* **6**, e19328 (2011).
13. Oliver, R. P. & Solomon, P. S. New developments in pathogenicity and virulence of necrotrophs. *Curr. Opin. Plant Biol.* **13**, 415–419 (2010).
14. Meyers, B. C., Kozik, A., Griego, A., Kuang, H. & Michelmore, R. W. Genome-wide analysis of NBS-LRR-encoding genes in *Arabidopsis*. *Plant Cell* **15**, 809–834 (2003).
15. Goff, S. A. *et al.* A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). *Science* **296**, 92–100 (2002).
16. Lukasik, E. & Takken, F. L. W. STANDing strong, resistance proteins instigators of plant defence. *Curr. Opin. Plant Biol.* **12**, 427–436 (2009).
17. Maekawa, T., Kufer, T. A. & Schulze-Lefert, P. NLR functions in plant and animal immune systems: so far and yet so close. *Nature Immunol.* **12**, 817–826 (2011).
18. Takken, F. L., Albrecht, M. & Tameling, W. I. Resistance proteins: molecular switches of plant defence. *Curr. Opin. Plant Biol.* **9**, 383–390 (2006).
19. Shirasu, K. The HSP90–SGT1 chaperone complex for NLR immune sensors. *Annu. Rev. Plant Biol.* **60**, 139–164 (2009).
20. Flor, H. H. Mutations in flax rust induced by ultraviolet radiation. *Science* **124**, 888–889 (1956).

21. Bakker, E. G., Toomajian, C., Kreitman, M. & Bergelson, J. A genome-wide survey of *R* gene polymorphisms in *Arabidopsis*. *Plant Cell* **18**, 1803–1818 (2006).
22. Jia, Y., McAdams, S. A., Bryan, G. T., Hershey, H. P. & Valent, B. Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. *EMBO J.* **19**, 4004–4014 (2000).
23. Deslandes, L. *et al.* Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. *Proc. Natl Acad. Sci. USA* **100**, 8024–8029 (2003).
24. Dodds, P. N. *et al.* Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. *Proc. Natl Acad. Sci. USA* **103**, 8888–8893 (2006).
25. Krasileva, K. V., Dahlbeck, D. & Staskawicz, B. J. Activation of an *Arabidopsis* resistance protein is specified by the in planta association of its leucine-rich repeat domain with the cognate oomycete effector. *Plant Cell* **22**, 2444–2458 (2010).
26. Mackey, D., Holt, B. F., 3rd, Wiig, A. & Dangl, J. L. RIN4 interacts with *Pseudomonas syringae* type III effector molecules and is required for RPM1-mediated resistance in *Arabidopsis*. *Cell* **108**, 743–754 (2002).
27. Kim, M. G. *et al.* Two *Pseudomonas syringae* type III effectors inhibit RIN4-regulated basal defense in *Arabidopsis*. *Cell* **121**, 749–759 (2005).
28. Liu, J., Elmore, J. M., Lin, Z. J. D. & Coaker, G. A receptor-like cytoplasmic kinase phosphorylates the host target RIN4, leading to the activation of a plant innate immune receptor. *Cell Host Microbe* **9**, 137–146 (2011).
The authors show that pathogen-induced phosphorylation of the host target RIN4 requires a host receptor-like kinase.
29. Chung, E. H. *et al.* Specific threonine phosphorylation of a host target by two unrelated type III effectors activates a host innate immune receptor in plants. *Cell Host Microbe* **9**, 125–136 (2011).
This study shows that R proteins perceive pathogen-induced phosphorylation of the host target RIN4 as a change in self and consequently activate plant immunity.
30. Axtell, M. J., Chisholm, S. T., Dahlbeck, D. & Staskawicz, B. J. Genetic and molecular evidence that the *Pseudomonas syringae* type III effector protein AvrRpt2 is a cysteine protease. *Mol. Microbiol.* **49**, 1537–1546 (2003).
31. Axtell, M. J. & Staskawicz, B. J. Initiation of RPS2-specified disease resistance in Arabidopsis is coupled to the AvrRpt2-directed elimination of RIN4. *Cell* **112**, 369–377 (2003).
32. Mackey, D., Belkhadir, Y., Alonso, J. M., Ecker, J. R. & Dangl, J. L. Arabidopsis RIN4 is a target of the type III virulence effector AvrRpt2 and modulates RPS2-mediated resistance. *Cell* **112**, 379–389 (2003).
33. Liu, J. *et al.* RIN4 functions with plasma membrane H^+-ATPases to regulate stomatal apertures during pathogen attack. *PLoS Biol.* **7**, e1000139 (2009).
This paper shows that RIN4 interacts with plasma membrane H^+-ATPases to prevent the opening of stomata, thereby restricting the entry of bacterial pathogens into the leaf.
34. Pritchard, L. & Birch, P. A systems biology perspective on plant–microbe interactions: biochemical and structural targets of pathogen effectors. *Plant Sci.* **180**, 584–603 (2011).
35. Mukhtar, M. S. *et al.* Independently evolved virulence effectors converge onto hubs in a plant immune system network. *Science* **333**, 596–601 (2011).
This study uses a large plant–pathogen protein interaction screen involving a high-throughput yeast two-hybrid assay to show that pathogen effectors target a relatively small set of interconnected host signalling hubs, some of which seem to be monitored by immune receptors.
36. Ting, J. P. Y., Willingham, S. B. & Bergstralh, D. T. NLRs at the intersection of cell death and immunity. *Nature Rev. Immunol.* **8**, 372–379 (2008).
37. Hatsugai, N. *et al.* A novel membrane fusion-mediated plant immunity against bacterial pathogens. *Genes Dev.* **23**, 2496–2506 (2009).
38. Coll, N. S. *et al.* Arabidopsis type I metacaspases control cell death. *Science* **330**, 1393–1397 (2010).
39. Wang, W. *et al.* Timing of plant immune responses by a central circadian regulator. *Nature* **470**, 110–114 (2011).
This study identifies new genes that are involved in R gene-mediated resistance against downy mildew in *A. thaliana* and shows that they are under circadian control, which enables plants to anticipate the pathogen's infection cycle.
40. Yu, I. C., Parker, J. & Bent, A. F. Gene-for-gene disease resistance without the hypersensitive response in *Arabidopsis dnd1* mutant. *Proc. Natl Acad. Sci. USA* **95**, 7819–7824 (1998).
41. Yu, I., Fengler, K. A., Clough, S. J. & Bent, A. F. Identification of *Arabidopsis* mutants exhibiting an altered hypersensitive response in gene-for-gene disease resistance. *Mol. Plant Microbe Interact.* **13**, 277–286 (2000).
42. Bomblies, K. *et al.* Autoimmune response as a mechanism for a Dobzhansky–Muller-type incompatibility syndrome in plants. *PLoS Biol.* **5**, e236 (2007).
The authors reveal that hybrid necrosis might result from auto-activation of an R protein in intraspecific *A. thaliana* hybrids.
43. Alcázar, R., García, A. V., Parker, J. E. & Reymond, M. Incremental steps toward incompatibility revealed by *Arabidopsis* epistatic interactions modulating salicylic acid pathway activation. *Proc. Natl Acad. Sci. USA* **106**, 334–339 (2009).
44. Bomblies, K. & Weigel, D. Hybrid necrosis: autoimmunity as a potential gene-flow barrier in plant species. *Nature Rev. Genet.* **8**, 382–393 (2007).
45. Jeuken, M. J. W. *et al. Rin4* causes hybrid necrosis and race-specific resistance in an interspecific lettuce hybrid. *Plant Cell* **21**, 3368–3378 (2009).
This paper shows that hybrid necrosis can also be caused by the effector-targeted host protein RIN4 in interspecific lettuce hybrids.
46. Alcázar, R. *et al.* Natural variation at Strubbelig receptor kinase 3 drives immune-triggered incompatibilities between *Arabidopsis thaliana* accessions. *Nature Genet.* **42**, 1135–1139 (2010).
47. Li, X., Clarke, J. D., Zhang, Y. & Dong, X. Activation of an EDS1-mediated R-gene pathway in the *snc1* mutant leads to constitutive, NPR1-independent pathogen resistance. *Mol. Plant Microbe Interact.* **14**, 1131–1139 (2001).
48. Shirano, Y., Kachroo, P., Shah, J. & Klessig, D. F. A gain-of-function mutation in an *Arabidopsis* Toll interleukin1 receptor–nucleotide binding site–leucine-rich repeat type R gene triggers defense responses and results in enhanced disease resistance. *Plant Cell* **14**, 3149–3162 (2002).
49. Oldroyd, G. E. & Staskawicz, B. J. Genetically engineered broad-spectrum disease resistance in tomato. *Proc. Natl Acad. Sci. USA* **95**, 10300–10305 (1998).
50. Stokes, T. L., Kunkel, B. N. & Richards, E. J. Epigenetic variation in *Arabidopsis* disease resistance. *Genes Dev.* **16**, 171–182 (2002).
51. Li, Y. *et al.* SRFR1 negatively regulates plant NB-LRR resistance protein accumulation to prevent autoimmunity. *PLoS Pathog.* **6**, e1001111 (2010).
52. Kim, S. H. *et al.* The *Arabidopsis* resistance-like gene *SNC1* is activated by mutations in *SRFR1* and contributes to resistance to the bacterial effector AvrRps4. *PLoS Pathog.* **6**, e1001172 (2010).
53. Kwon, S. I., Kim, S. H., Bhattacharjee, S., Noh, J. J. & Gassmann, W. *SRFR1*, a suppressor of effector-triggered immunity, encodes a conserved tetratricopeptide repeat protein with similarity to transcriptional repressors. *Plant J.* **57**, 109–119 (2009).
54. Cheng, Y. T. *et al.* Stability of plant immune-receptor resistance proteins is controlled by SKP1–CULLIN1–F-box (SCF)-mediated protein degradation. *Proc. Natl Acad. Sci. USA* **108**, 14694–14699 (2011).
55. Mishina, T. E. & Zeier, J. Pathogen-associated molecular pattern recognition rather than development of tissue necrosis contributes to bacterial induction of systemic acquired resistance in *Arabidopsis*. *Plant J.* **50**, 500–513 (2007).
56. Durrant, W. E. & Dong, X. Systemic acquired resistance. *Annu. Rev. Phytopathol.* **42**, 185–209 (2004).
57. Yalpani, N., Silverman, P., Wilson, T., Kleier, D. A. & Raskin, I. Salicylic acid is a systemic signal and an inducer of pathogenesis-related proteins in virus-infected tobacco. *Plant Cell* **3**, 809–818 (1991).
58. Gaffney, T. *et al.* Requirement of salicylic acid for the induction of systemic acquired resistance. *Science* **261**, 754–756 (1993).
59. Vernooij, B. *et al.* Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. *Plant Cell* **6**, 959–965 (1994).
60. Park, S.-W., Kaimoyo, E., Kumar, D., Mosher, S. & Klessig, D. F. Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. *Science* **318**, 113–116 (2007).
61. Attaran, E., Zeier, T. E., Griebel, T. & Zeier, J. Methyl salicylate production and jasmonate signaling are not essential for systemic acquired resistance in *Arabidopsis*. *Plant Cell* **21**, 954–971 (2009).
62. Maldonado, A. M., Doerner, P., Dixon, R. A., Lamb, C. J. & Cameron, R. K. A putative lipid transfer protein involved in systemic resistance signalling in *Arabidopsis*. *Nature* **419**, 399–403 (2002).
63. Truman, W., Bennett, M. H., Kubigsteltig, I., Turnbull, C. & Grant, M. *Arabidopsis* systemic immunity uses conserved defense signaling pathways and is mediated by jasmonates. *Proc. Natl Acad. Sci. USA* **104**, 1075–1080 (2007).
64. Chaturvedi, R. *et al.* Plastid omega3-fatty acid desaturase-dependent accumulation of a systemic acquired resistance inducing activity in petiole exudates of *Arabidopsis thaliana* is independent of jasmonic acid. *Plant J.* **54**, 106–117 (2008).
65. Nandi, A., Welti, R. & Shah, J. The *Arabidopsis thaliana* dihydroxyacetone phosphate reductase gene *SUPPRESSSOR OF FATTY ACID DESATURASE DEFICIENCY1* is required for glycerolipid metabolism and for the activation of systemic acquired resistance. *Plant Cell* **16**, 465–477 (2004).
66. Chanda, B. *et al.* Glycerol-3-phosphate is a critical mobile inducer of systemic immunity in plants. *Nature Genet.* **43**, 421–427 (2011).
This study provides compelling evidence that glycerol-3-phosphate and the lipid-transfer protein DIR1 together form an essential mobile signal for the induction of systemic immunity in plants.
67. Jung, H. W., Tschaplinski, T. J., Wang, L., Glazebrook, J. & Greenberg, J. T. Priming in systemic plant immunity. *Science* **324**, 89–91 (2009).
68. Liu, P. P., von Dahl, C. C., Park, S. W. & Klessig, D. F. Interconnection between methyl salicylate and lipid-based long-distance signaling during the development of systemic acquired resistance in Arabidopsis and tobacco. *Plant Physiol.* **155**, 1762–1768 (2011).
69. White, R. F. Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. *Virology* **99**, 410–412 (1979).
70. Wang, D., Amornsiripanitch, N. & Dong, X. A genomic approach to identify regulatory nodes in the transcriptional network of systemic acquired resistance in plants. *PLoS Pathog.* **2**, e123 (2006).
71. Sugano, S. *et al.* Role of OsNPR1 in rice defense program as revealed by genome-wide expression analysis. *Plant Mol. Biol.* **74**, 549–562 (2010).
72. Netea, M. G., Quintin, J. & van der Meer, J. W. M. Trained immunity: a memory for innate host defense. *Cell Host Microbe* **9**, 355–361 (2011).
73. Beckers, G. J. M. *et al.* Mitogen-activated protein kinases 3 and 6 are required for full priming of stress responses in *Arabidopsis thaliana*. *Plant Cell* **21**, 944–953 (2009).
This paper shows that priming of plant stress responses requires the enhanced accumulation of MPK3 and MPK6 proteins.
74. Mou, Z., Fan, W. & Dong, X. Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. *Cell* **113**, 935–944 (2003).
75. Wang, S., Durrant, W. E., Song, J., Spivey, N. W. & Dong, X. *Arabidopsis* BRCA2 and RAD51 proteins are specifically involved in defense gene transcription during plant immune responses. *Proc. Natl Acad. Sci. USA* **107**, 22716–22721 (2010).
76. Song, J. *et al.* DNA repair proteins are directly involved in regulation of gene expression during plant immune response. *Cell Host Microbe* **9**, 115–124 (2011).
77. Alvarez, M. E., Nota, F. & Cambiagno, D. A. Epigenetic control of plant immunity. *Mol. Plant Pathol.* **11**, 563–576 (2010).
78. Jaskiewicz, M., Conrath, U. & Peterhansel, C. Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. *EMBO Rep.* **12**, 50–55 (2011).
This article shows that priming of plant immune-related genes correlates with specific chromatin modifications at their promoters.
79. Baumgarten, A., Cannon, S., Spangler, R. & May, G. Genome-level evolution of resistance genes in *Arabidopsis thaliana*. *Genetics* **165**, 309–319 (2003).
80. Yi, H. & Richards, E. J. Gene duplication and hypermutation of the pathogen *Resistance* gene *SNC1* in the *Arabidopsis bal* variant. *Genetics* **183**, 1227–1234 (2009).

81. Boyko, A. *et al.* Transgenerational changes in the genome stability and methylation in pathogen-infected plants: (virus-induced plant genome instability). *Nucleic Acids Res.* **35**, 1714–1725 (2007).
82. McClintock, B. The significance of responses of the genome to challenge. *Science* **226**, 792–801 (1984).
83. Molinier, J., Ries, G., Zipfel, C. & Hohn, B. Transgeneration memory of stress in plants. *Nature* **442**, 1046–1049 (2006).
This article shows that the progeny of parental plants that were exposed to MAMPs maintain increased levels of somatic homologous recombination even in the absence of pathogen stress, suggesting a transgenerational memory of immunity.
84. Lucht, J. M. *et al.* Pathogen stress increases somatic recombination frequency in *Arabidopsis*. *Nature Genet.* **30**, 311–314 (2002).
85. Mosher, R. A., Durrant, W. E., Wang, D., Song, J. & Dong, X. A comprehensive structure–function analysis of *Arabidopsis* SNI1 defines essential regions and transcriptional repressor activity. *Plant Cell* **18**, 1750–1765 (2006).
86. Durrant, W. E., Wang, S. & Dong, X. *Arabidopsis* SNI1 and RAD51D regulate both gene transcription and DNA recombination during the defense response. *Proc. Natl Acad. Sci. USA* **104**, 4223–4227 (2007).
87. Walbot, V. Sources and consequences of phenotypic and genotypic plasticity in flowering plants. *Trends Plant Sci.* **1**, 27–32 (1996).
88. Ries, G. *et al.* Elevated UV-B radiation reduces genome stability in plants. *Nature* **406**, 98–101 (2000).
89. Pecinka, A. *et al.* Transgenerational stress memory is not a general response in Arabidopsis. *PLoS ONE* **4**, e5202 (2009).
90. Boyko, A. *et al.* Transgenerational adaptation of *Arabidopsis* to stress requires DNA methylation and the function of Dicer-like proteins. *PLoS ONE* **5**, e9514 (2010).
91. Lister, R. *et al.* Highly integrated single-base resolution maps of the epigenome in *Arabidopsis*. *Cell* **133**, 523–536 (2008).
92. Boller, T. & Felix, G. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu. Rev. Plant Biol.* **60**, 379–406 (2009).
93. Rast, J. P., Smith, L. C., Loza-Coll, M., Hibino, T. & Litman, G. W. Genomic insights into the immune system of the sea urchin. *Science* **314**, 952–956 (2006).
94. Lee, S. W. *et al.* A type I-secreted, sulfated peptide triggers XA21-mediated innate immunity. *Science* **326**, 850–853 (2009).
95. Lee, S. W., Han, S. W., Bartley, L. E. & Ronald, P. C. Unique characteristics of *Xanthomonas oryzae* pv. *oryzae* AvrXa21 and implications for plant innate immunity. *Proc. Natl Acad. Sci. USA* **103**, 18395–18400 (2006).
96. Han, S. W., Lee, S. W. & Ronald, P. C. Secretion, modification, and regulation of Ax21. *Curr. Opin. Microbiol.* **14**, 62–67 (2011).
97. Gómez-Gómez, L. & Boller, T. FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*. *Mol. Cell* **5**, 1003–1011 (2000).
98. Robatzek, S., Chinchilla, D. & Boller, T. Ligand-induced endocytosis of the pattern recognition receptor FLS2 in *Arabidopsis*. *Genes Dev.* **20**, 537–542 (2006).
99. Chinchilla, D. *et al.* A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature* **448**, 497–500 (2007).
100. Heese, A. *et al.* The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. *Proc. Natl Acad. Sci.* USA **104**, 12217–12222 (2007).
101. Van Loon, L. C. & Van Kammen, A. Polyacrylamide disc electrophoresis of the soluble leaf proteins from *Nicotiana tabacum* var. "Samsun" and "Samsun NN". II. Changes in protein constitution after infection with tobacco mosaic virus. *Virology* **40**, 190–211 (1970).
102. Van Loon, L. C. & Van Strien, E. A. The families of pathogenesis-related proteins, their activities, and comparative analysis of *PR-1* type proteins. *Physiol. Mol. Plant Pathol.* **55**, 85–97 (1999).
103. Zhu, Q., Maher, E. A., Masoud, S., Dixon, R. A. & Lamb, C. Enhanced protection against fungal attack by constitutive co–expression of chitinase and glucanase genes in transgenic tobacco. *Nature Biotech.* **12**, 807–812 (1994).
104. Thomma, B. P. H. J. *et al.* Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. *Proc. Natl Acad. Sci. USA* **95**, 15107–15111 (1998).
105. Wang, D., Weaver, N. D., Kesarwani, M. & Dong, X. Induction of protein secretory pathway is required for systemic acquired resistance. *Science* **308**, 1036–1040 (2005).
106. Silverstein, K. A. T., Graham, M. A., Paape, T. D. & VandenBosch, K. A. Genome organization of more than 300 defensin-like genes in *Arabidopsis*. *Plant Physiol.* **138**, 600–610 (2005).
107. Spoel, S. H. & Loake, G. J. Redox-based protein modifications: the missing link in plant immune signalling. *Curr. Opin. Plant Biol.* **14**, 358–364 (2011).
108. Tada, Y. *et al.* S-nitrosylation and thioredoxins regulate conformational changes of NPR1 in plant innate immunity. *Science* **321**, 952–956 (2008).
109. Després, C. *et al.* The *Arabidopsis* NPR1 disease resistance protein is a novel cofactor that confers redox regulation of DNA binding activity to the basic domain/leucine zipper transcription factor TGA1. *Plant Cell* **15**, 2181–2191 (2003).
110. Lindermayr, C., Sell, S., Muller, B., Leister, D. & Durner, J. Redox regulation of the NPR1–TGA1 system of *Arabidopsis thaliana* by nitric oxide. *Plant Cell* **22**, 2894–2907 (2010).
111. Hoffmann, A., Levchenko, A., Scott, M. L. & Baltimore, D. The IκB–NF-κB signaling module: temporal control and selective gene activation. *Science* **298**, 1241–1245 (2002).
112. Ashall, L. *et al.* Pulsatile stimulation determines timing and specificity of NF-κB-dependent transcription. *Science* **324**, 242–246 (2009).
113. Nelson, D. E. *et al.* Oscillations in NF-κB signaling control the dynamics of gene expression. *Science* **306**, 704–708 (2004).
114. Spoel, S. H. *et al.* Proteasome-mediated turnover of the transcription coactivator NPR1 plays dual roles in regulating plant immunity. *Cell* **137**, 860–872 (2009).
115. Spoel, S. H., Tada, Y. & Loake, G. J. Post-translational modification as a tool for transcription reprogramming. *New Phytol.* **186**, 333–339 (2010).

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