Journal of Experimental Botany
doi:10.1093/jxb/eraa072 Advance Access Publication 15 February 2020

REVIEW PAPER

Reprogramming and remodeling: transcriptional and epigenetic regulation of salicylic acid-mediated plant defense

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Received 27 August 2019; Editorial decision 31 January 2020; Accepted 11 February 2020

Editor: Steven Spoel, University of Edinburgh, UK

Abstract

As a plant hormone, salicylic acid (SA) plays essential roles in plant defense against biotrophic and hemibiotrophic pathogens. Significant progress has been made in understanding the SA biosynthesis pathways and SA-mediated defense signaling networks in the past two decades. Plant defense responses involve rapid and massive transcriptional reprogramming upon the recognition of pathogens. Plant transcription factors and their co-regulators are critical players in establishing a transcription regulatory network and boosting plant immunity. A multitude of transcription factors and epigenetic regulators have been discovered, and their roles in SA-mediated defense responses have been reported. However, our understanding of plant transcriptional networks is still limited. As such, novel genomic tools and bioinformatic techniques will be necessary if we are to fully understand the mechanisms behind plant immunity. Here, we discuss current knowledge, provide an update on the SA biosynthesis pathway, and describe the transcriptional and epigenetic regulation of SA-mediated plant immune responses.

Keywords: Arabidopsis, epigenetic regulators, plant immunity, *Pseudomonas syringae*, salicylic acid, transcription factors.

Introduction

In their natural environment, plants are constantly exposed to various kinds of microbial communities, including pathogens such as fungi, oomycetes, viruses, bacteria, and nematodes. As a consequence, plants have developed a multilayer system of immune responses (Jones and Dangl, 2006). The first line of defense in this system is initiated by the recognition of pathogen-associated molecular patterns (PAMPs) via pattern recognition receptors (PRRs), resulting in PAMP-triggered immunity (PTI) (Dodds and Rathjen, 2010). One of the best characterized PAMPs is flg22, a conserved 22 amino acid peptide from flagellin protein, which is recognized by the FLS2 (FLAGELLIN-SENSITIVE 2) receptor in Arabidopsis (Gómez-Gómez and Boller, 2000). As a counter defense, pathogens deliver effectors into the plant cell to suppress PTI, resulting in effector-triggered susceptibility (ETS) (Jones and Dangl, 2006). Through coevolution with pathogens, plants have developed resistance (R) genes that encode nucleotide-binding and leucine-rich repeat (NB-LRR) proteins, such as RPM1, RPS2, and RPS4, which specifically recognize corresponding avirulence (Avr) proteins AvrB or AvrRPM1, AvrRpt2, and AvrRPS4, resulting in effector-triggered immunity (ETI) (Jones and Dangl, 2006). There are two major classes of NB-LRR proteins that are distinguished by the domains present at their N termini: the Toll and Interleukin-1 Receptor homology (TIR) domain and coiled-coil (CC) motif (Adachi *et al.*, 2019). ETI is accompanied by hypersensitive

cell death, which prevents the spread of infection by microbial pathogens (Jones and Dangl, 2006). Both PTI and ETI activate several signaling events such as the production of reactive oxygen species (ROS) and nitric oxide (NO), and influx of Ca^{2+}, as well as the induction of different protein kinases such as the mitogen-activated protein kinases (MAPKs) (Chen *et al.*, 2014; Wendehenne *et al.*, 2014; Bi and Zhou, 2017). The subsequent localized defense response triggers systemic acquired resistance (SAR) in distal leaves preventing or reducing further infection (Pieterse *et al.*, 2009). In plants, PTI, ETI, and SAR are associated with elevated levels of the phytohormone salicylic acid (SA, 2-hydroxybenzoic acid), a small phenolic compound produced by a wide range of prokaryotic and eukaryotic organisms including plants (An and Mou, 2011; Dempsey *et al.*, 2011).

Salicylic acid biosynthesis pathways

SA levels increase in both local and distal parts of the plant upon pathogen infection (Malamy *et al.*, 1990; Métraux *et al.*, 1990). Biosynthesis of SA was proposed to occur in plants via two separate pathways: the isochorismate (IC) and the phenylalanine ammonia-lyase (PAL) (Dempsey *et al.*, 2011). Both pathways originate from chorismate, which is the end product of the shikimate pathway.

Isochorismate synthase (ICS) catalyses the conversion of chorismite to isochorismate (Wildermuth *et al.*, 2001; Strawn *et al.*, 2007; Garcion *et al.*, 2008). In some bacteria, the conversion of isochorismate to SA is catalysed by an isochorismate pyruvate lyase (IPL) (Gaille *et al.*, 2002). However, even after decades of study, an IPL homolog in plants has yet to be identified. Surprisingly, it was demonstrated recently that plants synthesize SA from isochorismate through a unique pathway (Chen *et al.*, 2019*a*; Rekhter *et al.*, 2019; Torrens-Spence *et al.*, 2019). In the chloroplast, ICS1 converts chorismate into isochorismate, which is then exported to the cytosol by a MATE transporter family protein ENHANCED DISEASE SUSCEPTIBILITY 5 (EDS5) (Nawrath *et al.*, 2002; Rekhter *et al.*, 2019; Torrens-Spence *et al.*, 2019). In the cytosol, AVRPPHB SUSCEPTIBLE 3 (PBS3) catalyses the conjugation of L-glutamate to isochorismate producing isochorismate-9-glutamate (Fig. 1) (Rekhter *et al.*, 2019; Torrens-Spence *et al.*, 2019). The final product, SA, is then synthesized from two different pathways. In one, SA is synthesized by spontaneous decay from isochorismate-9-glutamate without the use of an enzyme (Fig. 1) (Rekhter *et al.*, 2019; Torrens-Spence *et al.*, 2019). The other is an accelerated pathway in which ENHANCED PSEUDOMONAS SUSCEPTIBILITY 1 (EPS1) functions as an isochorismoyl-glutamate pyruvoyl-glutamate lyase (IPGL) cleaving *N*-pyruvoyl-L-glutamate from isochorismate-9-glutamate to produce SA. However, this second pathway only occurs in *Brassica* family plants (Fig. 1) (Torrens-Spence *et al.*, 2019). Together, PBS3 and EPS1 complete a new SA biosynthesis pathway from isochorismate in plants (Chen *et al.*, 2019*a*; Rekhter *et al.*, 2019; Torrens-Spence *et al.*, 2019), closing a significant knowledge gap.

Phenylalanine ammonia-lyase (PAL) converts phenylalanine to *trans*-cinnamic acid and ammonia via a non-oxidative deamination reaction (Rohde *et al.*, 2004). *trans*-Cinnamic acid is then converted to SA via two possible intermediates: *ortho*-coumaric acid or benzoic acid (BA) (Dempsey *et al.*, 2011). Genetic evidence supports an essential role of *PAL* in Arabidopsis SA biosynthesis. Arabidopsis contains four *PAL* genes (*PAL1–PAL4*), mutation of which results in a 90% loss of PAL activity (Huang *et al.*, 2010). The quadruple mutants accumulated 70% less SA compared with wild-type plants at basal level (Huang *et al.*, 2010). Similarly, SA content in the mutants was 40% lower than that in wild-type plants when challenged with an avirulent *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) strain carrying *avrRpt2*, indicating that *PAL* genes are also important for SA biosynthesis during the pathogen infection in Arabidopsis (Huang *et al.*, 2010). In contrast, a previous study showed that ICS1 contributes to 90–95% of pathogen-induced SA biosynthesis in Arabidopsis (Wildermuth *et al.*, 2001). Further studies will be required to resolve this discrepancy.

Important regulators of salicylic acid accumulation: EDS1, PAD4, SAG101, and NDR1

Over the past three decades, several defense-associated signaling genes that act upstream of SA have been revealed, such as ENHANCED DISEASE SUSCEPTIBILITY1 (EDS1), PHYTOALEXIN DEFICIENT4 (PAD4), SENESCENCE-ASSOCIATED GENE 101 (SAG101), and NON RACE-SPECIFIC DISEASE RESISTANCE 1 (NDR1) (Vlot *et al.*, 2009; Dempsey *et al.*, 2011; Qi *et al.*, 2018). Arabidopsis EDS1 is a lipase-like protein that confers resistance to biotrophic, hemibiotrophic, and non-host pathogens (Parker *et al.*, 1996; Aarts *et al.*, 1998; Falk *et al.*, 1999; Wiermer *et al.*, 2005; Moreau *et al.*, 2012). SA accumulation was abolished entirely in *eds1-2* mutants challenged with *Pst* DC3000 with or without the avirulence gene *avrRPS4* but not *avrRPM1* (Feys *et al.*, 2001), suggesting that EDS1 is required for avrRPS4-induced SA accumulation in Arabidopsis. It was shown that EDS1 is shuttled between the nucleus and cytoplasm, a function necessary for a complete innate immune response in plants (García *et al.*, 2010). Yeast two-hybrid screening of EDS1 interacting proteins resulted in the identification of PAD4, a lipase-like protein that is also important for SA signaling (Feys *et al.*, 2001). The *PAD4* gene was previously shown to be required for the synthesis of camalexin, which functions as a phytoalexin, in response to infection by the virulent bacterial pathogen *P. s.* pv. *maculicola* ES4326 (*Psm* ES4326) (Glazebrook *et al.*, 1997). In *pad4* mutants, SA synthesis was found to be reduced and delayed compared with wild-type plants in response to *Psm* ES4326 carrying *avrRpt2* (Zhou *et al.*, 1998). SAG101, another EDS1 interacting protein was discovered using a proteomic approach (Feys *et al.*, 2005). In *sag101* mutants, SA accumulation is reduced during the infection by *Fusarium graminearum* and cold stress (Chen *et al.*, 2015; Makandar *et al.*, 2015). EDS1 and PAD4 are present in the nucleus and cytoplasm,

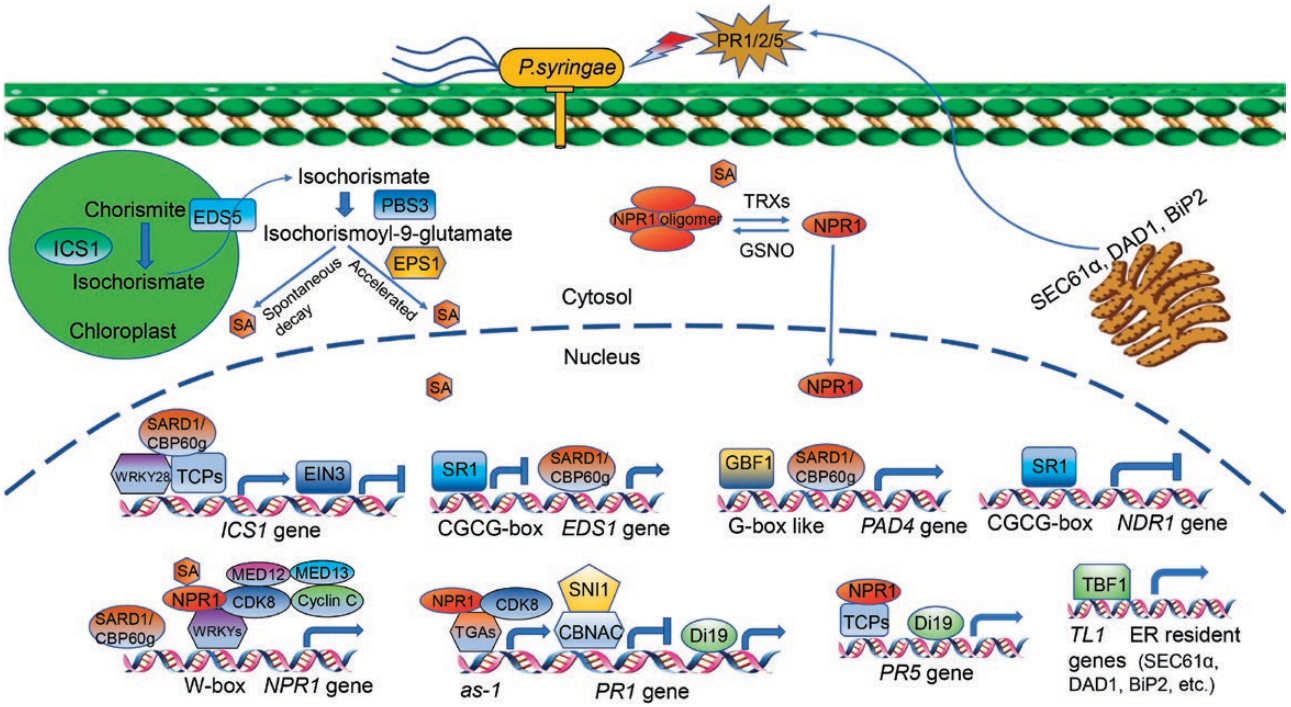

Fig. 1. Transcriptional regulation of salicylic acid-mediated plant immune responses. Upon pathogen infection, SA biosynthesis is initiated in the chloroplast. ICS1 converts chorismite to isochorismate, which is exported to the cytosol by EDS5. In the cytosol, PBS3 catalyses the conjugation of L-glutamate to isochorismate, creating isochorismate-9-glutamate. Next, SA is synthesized from L-glutamate via two separate pathways. In the spontaneous decay pathway, SA is produced from isochorismate-9-glutamate without any enzyme. In the accelerated pathway, EPS1 in *Brassica* family plants functions as an isochorismoyl-glutamate A pyruvoyl-glutamate lyase (IPGL) cleaving *N*-pyruvoyl-L-glutamate from isochorismate-9-glutamate to produce SA. SA biosynthesis is tightly controlled by several genes. SARD1 and CBP60g have been found to bind to the promoters of many genes including *ICS1*, *EDS1*, *NPR1*, and *PAD4*, but not *NDR1*. SR1 binds to the CGCG-box of these promoters and functions as a negative regulator of *NDR1* and *EDS1*. WRKY28, TCPs, and SARD1/CBP60g form a protein complex that positively regulates *ICS1* gene expression, whereas EIN3 acts conversely as a negative regulator. GBF1 binds to the G-box-like motif on the *PAD4* gene positively regulating its expression. SA promotes *NPR1* gene expression through the promotion of the interaction between WRKY transcription factors and NPR1, which recruits CDK8 to the W-box of the *NPR1* promoter facilitating its gene expression. The mediators of the CDK8 kinase module are also involved in *NPR1* gene expression. A high level of SA induced by pathogen infection causes the reduction of NPR1 oligomers to monomers, which enter the nucleus to activate downstream NPR1-dependent genes, including *PR* and ER-resident genes. *PR* genes represent the hallmark of defense responses under the control of *NPR1* and TGAs. SNI1 negatively regulates *PR1* gene expression through interaction with CBNAC, a transcription repressor of *PR1*. Di19 positively regulates *PR* genes, including *PR1*, *PR2*, and *PR5*. NPR1 interacts with TCP transcription factors, including TCP8, TCP14, and TCP15, which bind to the TCP binding site of the *PR5* promoter to promote *PR5* expression. *PR1* is also tightly controlled by epigenetic regulation. SA induces elevated levels of H3Ac, H4Ac, H3K4me2, and H3K4me3 at the *PR1* promoter. HAC–NPR1–TGA form a protein complex that binds to the *as-1* sequence of the *PR1* promoter to positively regulate *PR1* gene expression. HAC1 and HAC5 facilitate acetylation of Histone 3 on the *PR1* promoter. HDA6 and HDA19 function as histone deacetylases negatively regulating *PR1* gene expression through WRKY transcription factors. JMJ27 functions as an H3K9 demethylase that positively regulates *PR1* gene expression. TBF1 recognizes the *TL1 cis*-element on the promoter of ER-resident genes, such as *SEC61α*, *DAD1*, and *BiP2*, to control their expression. ER-resident genes control the secretion of PR proteins into the apoplast to combat pathogens.

whereas SAG101 preferentially localizes to the nucleus (Zhu *et al.*, 2011). EDS1 forms separate protein complexes with PAD4 and SAG101 at different subcellular locations and is required for PAD4 and SAG101 protein stability. Apart from promoting SA biosynthesis, the EDS1 and PAD4 protein complex maintains important SA-related resistance programs thereby increasing the effectiveness of the innate immune system (Cui *et al.*, 2017). EDS1 and PAD4 work in parallel with SA to protect against perturbations to SA in Arabidopsis basal and effector-triggered immunity (Cui *et al.*, 2017).

While EDS1 is required for resistance mediated by RPS4, a TIR-NB-LRR type R protein, NDR1 plays a vital role in resistance responses initiated by the CC-NB-LRR (coiled-coil–nucleotide binding–leucine-rich repeat) class of R proteins (Aarts *et al.*, 1998). NDR1 was first identified by the screening of mutants susceptible to *Pst* DC3000 carrying *avrB* gene (Century *et al.*, 1995). The *ndr1-1* mutant is SAR defective. SA accumulation, *PATHOGENESIS-RELATED GENE 1* (*PR1*) gene expression, and ROS production are impaired in the *ndr1-1* mutant in response to *Pseudomonas syringae* strains carrying *avrRpt2* (Shapiro and Zhang, 2001). NDR1 is localized to the plasma membrane via a C-terminal glycosylphosphatidylinositol (GPI) anchor, which allows NDR1 to act as an intercellular transducer of pathogen signals (Coppinger *et al.*, 2004). NDR1 undergoes several post-translational modifications, including carboxy-terminal processing and N-linked glycosylation (Coppinger *et al.*, 2004). Using a yeast two-hybrid screening, RIN4 was identified as an NDR1 interacting partner (Day *et al.*, 2006). RIN4–NDR1 interaction occurs in the cytoplasm and is required for activation of resistance signaling after infection by *Pst* DC3000 carrying *avrRpt2* gene (Day *et al.*, 2006). NDR1 was predicted to have a high degree

of structural similarity to Arabidopsis integrin-like protein LATE EMBRYOGENESIS ABUNDANT14 (Knepper *et al.*, 2011). This led to the identification of the role of NDR1 in preventing fluid loss and maintaining cell integrity (Knepper *et al.*, 2011).

Transcription factors involved in the regulation of salicylic acid biosynthesis and/or accumulation

A large group of transcription factors has been found to regulate the expression of important genes involved in SA biosynthesis and/or accumulation. Electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP) assay showed that WRKY28 binds to the *ICS1* promoter positioned −445 and −460 base pairs upstream of the transcription start site (van Verk *et al.*, 2011). Mutation of the binding site demonstrated that WRKY28 binding sites are essential for the activation of the *ICS1* promoter (van Verk *et al.*, 2011). In addition, SARD1 and CBP60g were also identified as crucial regulators of *ICS1* induction and SA biosynthesis (Zhang *et al.*, 2010). After pathogen infection, both proteins are targeted to the promoter region of *ICS1*. Pathogen-induced *ICS1* up-regulation and SA biosynthesis are compromised in *sard1-1 cbp60g-1* double mutants, resulting in compromised basal resistance and loss of SAR. A yeast one-hybrid system was used to screen for regulators of *ICS1*, leading to the identification of the TCP family transcription factor AtTCP8 (Wang *et al.*, 2015). TCP8 binds to the *ICS1* promoter at the TCP binding site *in vitro* and *in vivo*. *Tcp8 tcp9* double mutants show a significant reduction of *ICS1* transcription (Wang *et al.*, 2015). Furthermore, both TCP8 and TCP20 interact with SARD1, WRKY28, and NAC019, and TCP9 and TCP19 interact with SARD1 and WRKY28, suggesting that a transcription complex including TCP proteins is involved in the orchestrated regulation of *ICS1* expression (Fig. 1) (Wang *et al.*, 2015).

Negative regulators have also been found to suppress *ICS1* gene expression. The transcription factors Ethylene Insensitive 3 (EIN3) and EIN3-Like 1 (EIL1) have long been known to positively regulate ethylene-dependent responses (Guo and Ecker, 2003). However, increasing evidence suggests that EIN3 and EIL1 are important regulators of PTI and SA-mediated plant defense (Chen *et al.*, 2009; Boutrot *et al.*, 2010). Plants lacking *EIN3* and *EIL1* display enhanced PTI and are more resistant to both virulent and avirulent *Pseudomonas syringae* (Chen *et al.*, 2009). The *ein3-1 eil1-1* double mutants constitutively accumulate SA in the absence of pathogen stress (Chen *et al.*, 2009). Further analyses showed that EIN3 specifically binds to P5 fragments (−117 to −324 bp upstream of translational start site) of the *ICS1* promoter sequence *in vitro* and *in vivo* (Fig. 1) (Chen *et al.*, 2009). Thus, EIN3 and EIL1 appear to be key regulators at the intersection of ethylene and SA signaling by directly targeting *ICS1* to down-regulate SA-mediated defense.

Arabidopsis signal responsive (AtSR) proteins (also known as CAMTA3), a class of Ca^{2+}/calmodulin-binding transcription factors, play an essential role in regulating *EDS1* expression (Du *et al.*, 2009). In an EMSA, it was shown that the SR1 DNA-binding domain binds to the *EDS1* promoter at CGCG box. This binding was confirmed by a ChIP assay. *EDS1* promoter activity in *sr1-1* mutants is increased compared with that of Col-0, indicating that AtSR1 negatively regulates *EDS1* (Fig. 1). In addition, SR1 plays a pivotal role in plant immunity by directly regulating *NDR1* (Nie *et al.*, 2012). SR1 directly binds the promoter region of *NDR1* to suppress its expression (Fig. 1).

Recent studies indicate that PBS3 functions as one of the most important proteins in SA biosynthesis (Rekhter *et al.*, 2019; Torrens-Spence *et al.*, 2019); however, the transcription of *PBS3* is not well understood. One study showed that Arabidopsis protoplasts overexpressing *WRKY46* display a 4-fold increase in levels of *PBS3* mRNA accumulation, suggesting that WRKY46 is a transcriptional activator of *PBS3*. However, there are no data confirming that WRKY46 directly binds to the *PBS3* promoter (van Verk *et al.*, 2011).

The expression of *PAD4* is regulated by G-BOX BINDING FACTOR 1 (GBF1) in an intron-dependent manner (Giri *et al.*, 2017). It was found that GBF1 binds to the G-box-like element in the intron of *PAD4*, which is enhanced upon pathogen infection (Fig. 1). In *gbf1* mutants, like *pad4* mutants, SA accumulation and *PR1* gene expression are compromised. Compared with wild-type plants, *GBF1* overexpression plants showed significantly higher levels of *PAD4* mRNA accumulation upon pathogen inoculation.

Perception of salicylic acid

Generally, it is understood that plant and animal hormones transduce their signals by binding to one or multiple receptors. In the early 1990s, after the discovery of the function of SA in plant immunity, the first SA binding protein was identified (Chen and Klessig, 1991). Since then, dozens of SA binding proteins have been discovered or characterized (Chen *et al.*, 1993; Du and Klessig, 1997; Slaymaker *et al.*, 2002; Kumar and Klessig, 2003; Fu *et al.*, 2012; Manohar *et al.*, 2014; Yuan *et al.*, 2017; Ding *et al.*, 2018). SA binding proteins play important roles in plant immunity. For instance, silencing of *NtSABP2*, which encodes a methyl salicylate esterase, suppresses local resistance to tobacco mosaic virus, induction of *PR1* gene expression by SA, and development of SAR (Kumar and Klessig, 2003). However, only NPR1, NPR3, and NPR4 are considered bona fide SA receptors (Fu *et al.*, 2012; Wu *et al.*, 2012; Ding *et al.*, 2018). Among all the NPRs that have been tested, NPR4 shows the highest binding affinity to SA (Fu *et al.*, 2012; Ding *et al.*, 2018).

In order to identify key components that function downstream of SA, several forward genetic screenings were implemented to identify the mutants that do not respond to SA or its active analogs. The *NONEXPRESSER OF PR GENES 1* (*npr1*) mutant was first identified during a screening of Arabidopsis mutants that were unable to activate the expression of *PR* genes or mount SAR (Cao *et al.*, 1994; Delaney *et al.*, 1995). NPR1 contains an N-terminal BTB/POZ domain, central ankyrin-repeats, and a C-terminal transcriptional

activation motif (Cao *et al.*, 1997; Rochon *et al.*, 2006). In the cytosol, NPR1 mainly exists as oligomers. Upon pathogen infection or SA treatment, NPR1 is reduced from an oligomeric state to a monomeric state and translocated to the nucleus to accomplish its function (Mou *et al.*, 2003). SA affects NPR1 function at two stages: first, it induces *NPR1* gene expression; second, SA promotes translocation of NPR1 into the nucleus. SA-induced NPR1 oligomer-to-monomer transition is catalysed by thioredoxins (Tada *et al.*, 2008). NPR1 is not only sensitive to redox changes but also regulated by several post-translational modifications, such as phosphorylation, ubiquitination, and sumoylation (Spoel *et al.*, 2009; Lee *et al.*, 2015; Saleh *et al.*, 2015; Chen *et al.*, 2017).

In contrast to NPR1, which positively regulates SA-mediated plant immunity, NPR3 and NPR4 function as negative regulators of plant defense (Zhang *et al.*, 2006; Fu *et al.*, 2012). It has been demonstrated that NPR3 and NPR4 function as adaptors of the Cullin3 ubiquitin 3 E3 ligase to mediate NPR1 and EDS1 degradation (Fu *et al.*, 2012; Chang *et al.*, 2019). Supporting this, it was shown that *npr3 npr4* double mutant accumulates a higher level of NPR1 and EDS1 proteins (Fu *et al.*, 2012; Chang *et al.*, 2019). In addition, NPR3 and NPR4 have been shown to facilitate the degradation of JASMONATE ZIM-DOMAIN (JAZ) proteins to promote ETI (Liu *et al.*, 2016). Ding *et al.* showed that NPR3 and NPR4 function as transcriptional co-repressors and SA inhibits their activities to promote plant defense gene expression (Ding *et al.*, 2018).

Transcriptional regulation of *NPR1* gene

NPR1 plays a pivotal role in plant immunity, but the regulation of *NPR1* gene expression has not been extensively studied. Only two transcription factors have been reported to regulate *NPR1* gene expression through binding to its promoter (Yu *et al.*, 2001; Chai *et al.*, 2014). WRKY18, a SA-induced protein, specifically recognizes the W-box motif in the *NPR1* promoter (Yu *et al.*, 2001). The *npr1* mutants containing an *NPR1* gene with a mutated W-box are unable to induce SA-dependent gene expression or resistance, indicating that the W-box motif in the *NPR1* promoter is essential for its gene expression (Yu *et al.*, 2001). It was found that several other *WRKY* genes are up-regulated in the presence of SA, suggesting that there may be additional WRKY family proteins involved in *NPR1* gene regulation (Yu *et al.*, 2001). More recently, it was shown that WRKY6 binds to the W-box of the *NPR1* promoter and is required for *NPR1* gene expression (Chai *et al.*, 2014).

Not only are transcription factors involved in the promotion of *NPR1* gene expression, but also NPR1 itself. It has been found that NPR1 is capable of binding to the W-box motif of its own promoter (Chen *et al.*, 2019*b*). Because NPR1 does not have a DNA binding domain, the binding of NPR1 to its own promoter must be mediated by transcription factors. Indeed, it has been shown that WRKY18 interacts with NPR1, an interaction that is enhanced by SA (Chen *et al.*, 2019*b*). Filling the gap between transcription factors and transcription machinery, Chen *et al.* found that CDK8 functions as a bridge between WRKY18 and RNA polymerase II. WRKY6 and WRKY18 interact with CDK8, which brings RNA polymerase II to *NPR1* promoters and coding regions, facilitating its expression (Chen *et al.*, 2019*b*). Furthermore, it has been shown that SA promotes the interaction between NPR1 and CDK8 as well, indicating that NPR1 recruits CDK8 to facilitate its own expression (Fig. 1) (Chen *et al.*, 2019*b*). Consistent with these findings, the expression of *NPR1* and *NPR1*-dependent defense genes, including *PR1*, is significantly reduced in *cdk8* mutants compared with wild-type plants (Chen *et al.*, 2019*b*).

Transcriptional regulation of *PR* genes

As a master immune regulator, NPR1 controls the expression of over 2000 genes (Wang *et al.*, 2006). However, NPR1 itself does not contain a known DNA binding domain. NPR1-mediated signaling requires interaction with other transcription factors. Yeast two-hybrid screening has revealed that NPR1 interacts with seven members of the TGACG-Binding (TGA) transcription factor family (Després *et al.*, 2000; Zhou *et al.*, 2000; Kim and Delaney, 2002; Boyle *et al.*, 2009). Interestingly, *in planta* protein–protein interaction assays showed that the interaction between NPR1 and TGA1 or TGA4 requires SA (Després *et al.*, 2003). The interaction between NPR1 and TGA2 has been detected in the absence of SA, but this interaction is known to be enhanced by SA (Fan and Dong, 2002). NPR1 is capable of amplifying the DNA binding activity of TGA proteins and thus affects the expression of *PR* genes (Fig. 1) (Després *et al.*, 2000). In the nucleus, NPR1 monomers interact with TGAs, which target the *activation sequence-1* (*as-1*) element of the *PR1* promoter (Zhou *et al.*, 2000). NPR1 promotes the expression of *PR5* through interaction with TCP15, which promotes the expression of *PR5* by directly binding to a TCP binding site within its promoter (Fig. 1) (Li *et al.*, 2018). Another transcription factor that positively regulates *PR* genes expression is Drought-induced 19 (Di19), which is a seven Cys2/His2-type zinc-finger protein (Liu *et al.*, 2013). Di19 exhibits transactivation activity in yeast and binds to the TACA(A/G)T element within the *PR1*, *PR2*, and *PR5* promoters in Arabidopsis (Liu *et al.*, 2013). It promotes the expression of *PR1*, *PR2*, and *PR5*, and the expression was enhanced by Di19 interacting protein CPK11 (Liu *et al.*, 2013).

Through genetic screening for suppressors of *npr1-1*, the suppressor of *npr1-1*, inducible 1 (*sni1*) mutant was identified (Li *et al.*, 1999). The *sni1 npr1* double mutants show near wild-type levels of *PR1* expression and resistance to pathogens (Li *et al.*, 1999). SNI1 is a leucine-rich nuclear protein that exerts its negative effect on *PR1* expression by association with *PR1* promoter at −816 and −573 under non-induced conditions (Pape *et al.*, 2010). In the presence of SA, the function of SNI1 and its target is inactivated (Pape *et al.*, 2010). Kim *et al.* demonstrated that CBNAC, a calmodulin-regulated NAC transcriptional repressor, is the target of SNI1 (Kim *et al.*, 2012). SNI1 interacts with CBNAC and enhances the binding of CBNAC

to the *PR1* promoter (Kim *et al.*, 2012). The *cbnac1* mutant displays enhanced resistance to *Pst* DC3000 and increased *PR1* expression (Kim *et al.*, 2012).

Transcriptional regulation of secretion-related genes

In addition to *PR* genes, NPR1 also directly controls the expression of genes governing protein secretory pathways (Wang *et al.*, 2005). These secretion-related genes include *SUPPRESSORS OF SECRETION-DEFECTIVE* (*Sec61*) α and *Sec61*β, which provide a channel for proteins to cross the ER (endoplasmic reticulum) membrane (Wang *et al.*, 2005). NPR1 also regulates genes encoding ER-resident chaperones, such as luminal binding protein (BiP2) and glucose-regulated protein 94 (GRP94), as well as co-chaperones such as defender against apoptotic death 1 (AtDAD1), calnexins (CNXs), calreticulins (CRTs), and protein disulfide isomerases (PDIs) (Wang *et al.*, 2005). In *sec61*α, *dad1*, and *bip2* single and *sec61*α *bip2* and *dad1 bip2* double mutants, benzothiadiazole (BTH)-induced PR1 secretion and disease resistance against *Psm* ES4326 were significantly reduced (Wang *et al.*, 2005). Using a MEME program, Wang *et al.* identified a consensus sequence, *TL1* (CTGAAGAAGAA), in the promoter regions of NPR1-responsive ER-resident genes (Wang *et al.*, 2005). Subsequent EMSA results showed that SA promotes the binding of nuclear protein extracts to *TL1* elements in wild type plants but not *npr1* mutants, suggesting that *TL1* is a *cis*-element involved in SA induction of secretion-related genes via NPR1 (Wang *et al.*, 2005).

To identify the transcription factors that bind to *TL1* cis-element (TBF), Pajerowska-Mukhtar *et al.* (2012) used the TFSEARCH database and found the heat-shock factor-like protein (HSF) was a potential target. The Arabidopsis genome contains 21 HSF-like genes, but only *HSF4* is strongly induced by BTH and *Psm* ES4326 (Pajerowska-Mukhtar *et al.*, 2012). Combining a yeast one-hybrid and an EMSA assay, it was found that HSF4 is the *TL1*-binding transcription factor TBF1 (Pajerowska-Mukhtar *et al.*, 2012). Using a ChIP assay, it was demonstrated that HSF4 binds to the *TL1* element in the *BiP2* promoter, a function induced by SA (Fig. 1) (Pajerowska-Mukhtar *et al.*, 2012). Interestingly, *NPR1* gene expression in *tbf1* mutants is reduced compared with Col-0 in response to SA (Pajerowska-Mukhtar *et al.*, 2012). The *NPR1* promoter contains a *TL1* element. Thus, TBF1 may directly regulate *NPR1* expression through a *TL1 cis*-element.

The function of Mediators in salicylic acid-mediated plant defense

An important feature of plant defense response is the massive reprogramming of gene expression (Gruner *et al.*, 2013). Recent studies have revealed a link between Mediators and plant immune transcriptional processes against bacterial and fungal pathogens (Wathugala *et al.*, 2012; An and Mou, 2013; Lai *et al.*, 2014; Zhu *et al.*, 2014). These Mediators are purported to function as a bridge between RNA polymerase II and DNA binding transcription factors (Kidd *et al.*, 2011). The Mediator complex consists of 20–30 subunits forming four subcomplexes: Head, Middle, Tail, and the cyclin-dependent kinase module (Jeronimo and Robert, 2017). Here, we discuss various Mediators that have been identified as essential regulators in SA signaling.

Mediator 14 (MED14) functions as a bridge between three (Head, Middle, and Tail) modules (Soutourina, 2018). The *med14* mutation strongly suppresses the expression of SA pathway genes, such as *PR1*, *NPR1*, *EDS1*, *PAD4*, *ICS1*, and *EDS5*, and reduces disease resistance to *Pst* DC3000 and *Pst* DC3000 *avrRpt2* (Zhang *et al.*, 2013). Microarray data show that *med14* mutation inhibits a large group of defense genes, including positive and negative SAR regulators (Zhang *et al.*, 2013).

Mediator 15 (MED15) and Mediator 16 (MED16) in the tail module of the Mediator complex positively regulate plant immunity. NRB4, an ortholog of MED15, was identified during a genetic screening for mutants that are insensitive to SA (Canet *et al.*, 2010). *NRB4* null mutants exhibit even more insensitivity to SA than *npr1* (Canet *et al.*, 2012). The *nrb4* mutants are more susceptible to *Pst* DC3000 but not to *Pst* DC3000 carrying *avrRpm1* compared with Col-0 (Canet *et al.*, 2012). SA-induced disease resistance and *PR1* expression are compromised in *nrb4* mutants as well (Canet *et al.*, 2012; Wang *et al.*, 2016). Another member of the tail module subunits, MED16, also known as SENSITIVE TO FREEZING6 (SFR6), was first identified in a screening for mutants that fail to acclimate to cold stress (Warren *et al.*, 1996). Later, another study identified a mutant, *insensitive to exogenous NAD*$^+$ (*ien1*)/*med16-1*, that was defective in *PR1* gene expression in response to exogenous NAD^+ (Zhang *et al.*, 2012). The *sfr6* and *med16-1* mutants are more susceptible to virulent or avirulent *Pseudomonas syringae* infection and accumulate less *PR1*, *PR2*, and *PR5* mRNA compared with Col-0 (Wathugala *et al.*, 2012; Zhang *et al.*, 2012). MED16 is an essential regulator of SAR. It was shown that mutation in *MED16* results in reduced NPR1 protein levels and completely compromised SAR (Zhang *et al.*, 2012). It is not clear how MED16 regulates NPR1 protein stability. This should be a focal point of future investigation.

The CDK8 kinase module is a conserved dissociable Mediator subcomplex that links to the RNA polymerase II (RNAPII) C-terminal domain (Tsai *et al.*, 2013). CDK8 contributes to the transcriptional regulation of genes involved in SA biosynthesis and the SA signaling pathway (Huang *et al.*, 2019). The expression of SA biosynthesis genes such as *ICS1* and *EDS5* is down-regulated in *cdk8* mutants under uninfected conditions (Huang *et al.*, 2019). As discussed earlier, Chen *et al.* found that CDK8 positively regulates the expression of *NPR1* and its target genes by connecting WRKY transcription factors to RNAPII (Chen *et al.*, 2019*b*). CDK8 also fine-tunes SA levels in plants that overaccumulate SA. It was shown that *CDK8* mutation suppresses the SA level in *calmodulin-binding transcription activator 1/2/3* (*camta1/2/3*) mutants (Huang *et al.*, 2019).

The *MED5* mutant *reduced epidermal fluorescence 4* (*ref4*)-*3* displays a dwarf phenotype and overaccumulates SA, which are not observed in *ref4-3 cdk8* double mutants (Mao *et al.*, 2019). Apart from CDK8, Mediator kinase module members MED12 and MED13 also play an important role in SA-mediated gene expression (Fig. 1). Arabidopsis *med12* and *med13* mutants accumulate significantly lower amounts of *NPR1* and *PR1* transcripts compared with Col-0 under SA treatment (Chen *et al.*, 2019*b*). SAR is compromised in *med12* and *med13* mutants as well (Chen *et al.*, 2019*b*; Huang *et al.*, 2019). Moreover, *med12* mutants show reduced SA levels and reduced *ICS1* and *EDS5* gene expression under uninfected conditions, indicating that MED12 contributes to SA biosynthesis (Huang *et al.*, 2019).

Epigenetic regulators involved in the transcriptional regulation of salicylic acid-mediated plant immune response

Salicylic acid and its active analogs induce chromatin modifications

Chromatin modification and remodeling were described as another layer of regulation for transcriptional reprogramming during SA-mediated plant immune responses (Alvarez *et al.*, 2010). SA accumulation induces changes in chromatin structure, and exogenous application of SA induces increased levels of H3Ac, H4Ac, H3K4me2, and H3K4me3 at the *PR1* promoter facilitating the expression of *PR1* gene (Fig. 2) (Mosher *et al.*, 2006; van den Burg and Takken, 2009).

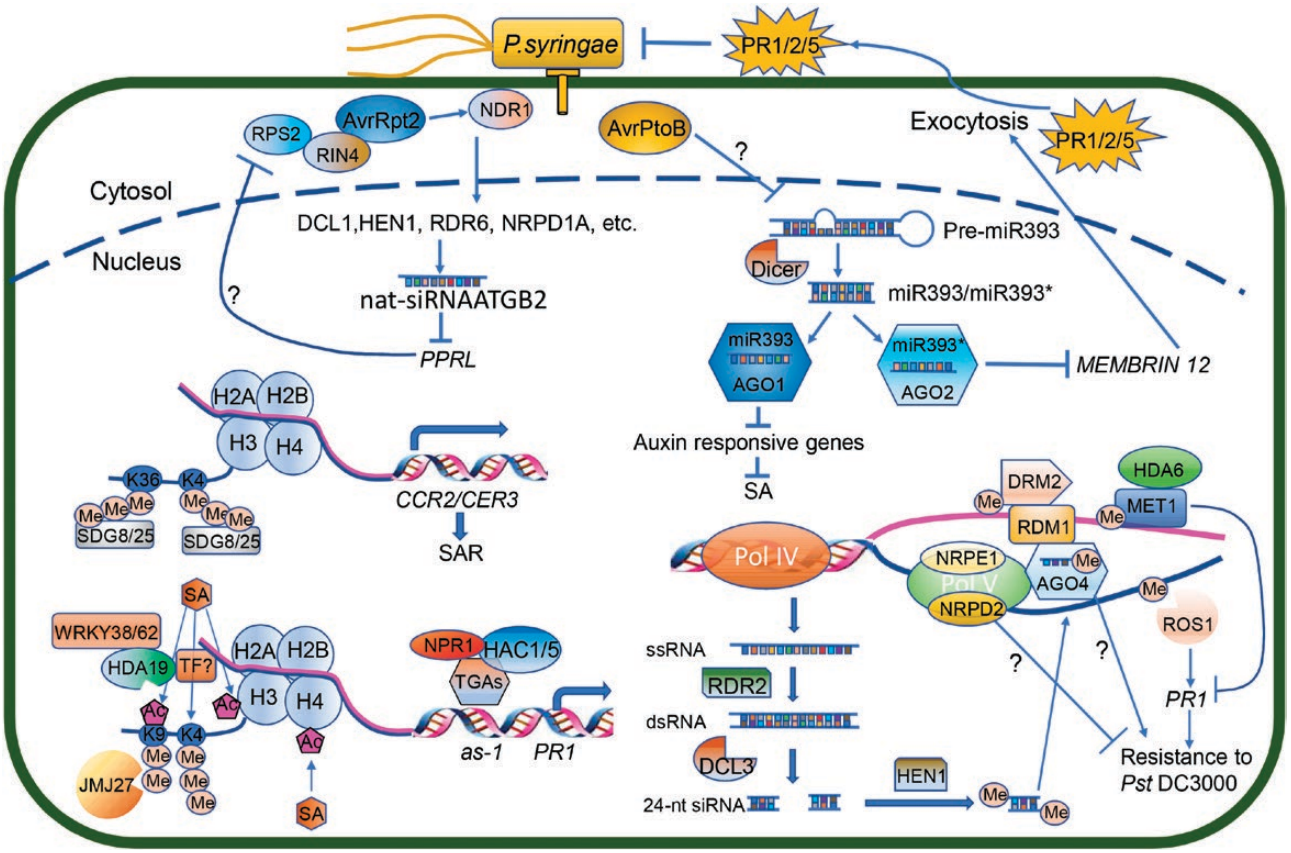

Fig. 2. Epigenetic regulation of salicylic acid-mediated plant immune responses. A pair of miRNA duplexes, miR393/miR393*, which were produced by dicer from Pre-miR393, function differently in plant immunity. miR393 was loaded into AGO1, resulting in the suppression of auxin-responsive genes, which are negative regulators in SA-mediated plant immunity. miR393*, on the other hand, was loaded into AGO2 to suppress the expression of *MEMBRIN 12*, which positively modulates the exocytosis of antimicrobial PR proteins. *Pseudomonas syringae* pv. *tomato* DC3000 delivers type III effector protein AvrPtoB into the plant cell causing the suppression of Pre-miR393 accumulation. The type III effector AvrRpt2 delivered by *Pseudomonas syringae* induces the accumulation of nat-siRNAATGB2, which suppresses the expression of *PPRL*, a negative regulator of RPS2-mediated plant immunity. The accumulation of nat-siRNAATGB2 is dependent on the resistance genes NDR1 and RPS2. DCL1, HEN1, RDR6, and NRPD1A are required for biosynthesis of nat-siRNAATGB2. SDG8 and SDG25 histone methyltransferases regulate H3K4 and H3K36 methylations on chromatin surrounding the *CCR2* and *CER3* locus. SA induces increased levels of H3Ac, H4Ac, H3K4me2, and H3K4me3 at the *PR1* promoter. The HAC1/5–NPR1–TGAs protein complex is recruited to the *PR* chromatin to activate *PR* transcription by histone acetylation. HDA19 binds to the promoter through unknown transcription factor(s), causing the deacetylation of H3K9. HDA19 interacts with WRKY38 and WRKY62, which negatively regulate plant immunity. JMJ27 is a histone demethylase that regulates the level of methylation of H3K9 at the *PR1* promoter. Proteins involved in RNA-directed DNA methylation pathways are also involved in SA-mediated plant immunity. Pol IV synthesizes siRNAs, which are subsequently transcribed by RDR2 to produce dsRNAs. The dsRNAs are processed by DCL3 into 24-nt siRNAs that are methylated at their 3′ ends by HEN1 and incorporated into AGO4, which is required for resistance to *Pst* DC3000. The two largest subunits of PolV, NRPE1 and NRPD2, suppress plant resistance to *Pst* DC3000. RDM1 mediates the interaction between AGO4 and DRM2, which is required for *de novo* DNA methylation. HDA6 interacts with MET1, which encodes a cytosine methyltransferase that negatively regulates the expression of *PR1*. ROS1, a DNA demethylase, positively regulates the expression of *PR1*. Ac, acetylation; Me, methylation.

NPR1 is required for H3Ac increase at the *PR1* promoter (Koornneef *et al.*, 2008). In addition, BTH treatment induces H3K4 trimethylation and dimethylation at the promoters of BTH-inducible WRKY transcription factors *WRKY6* and *WRKY53* (Jaskiewicz *et al.*, 2011).

Reversible histone acetylation regulates many aspects of plant defense

Histone deacetylases, a class of enzymes that remove acetyl groups from histone, allow the histones to wrap DNA more tightly, making the DNA less accessible to transcription factors. Studies have shown that histone deacetylases are involved in biotic and abiotic stress responses (Kim *et al.*, 2008; Chen *et al.*, 2010; Choi *et al.*, 2012; Buszewicz *et al.*, 2016; Zheng *et al.*, 2016; Wang *et al.*, 2017). Histone deacetylase 19 (HDA19) was found to interact with WRKY62 and WRKY38 (Kim *et al.*, 2008). *Hda19* mutants accumulate less *PR1* mRNA and are more susceptible to *Pst* DC3000 when compared with Col-0 (Kim *et al.*, 2008). However, an independent study performed by Choi *et al.* found that the expression of defense genes such as *PR1* and *PR2* and genes involved in SA accumulation such as *EDS1*, *EDS5*, and *ICS1* was increased in *hda19* mutants (Choi *et al.*, 2012). The different conclusions obtained from these independent studies may be due to differences in bacterial concentration used in the assay. The ChIP assay showed that levels of H3K9Ac in the *PR1* and *PR2* promoter regions were higher in *hda19* mutants than that in wild-type plants, indicating that *hda19* mutations may cause hyper-acetylation of histones at *PR* loci (Choi *et al.*, 2012). HDA19 regulates *PR* promoter activity by binding to its promoter via an unknown transcription factor(s) (Fig. 2). For this reason, it would be interesting to identify the HDA19-recruiting transcription factors that negatively regulate *PR* genes.

Another histone deacetylase involved in epigenetic regulation of SA-mediated plant immune response is histone deacetylase 6 (HDA6). Wang *et al.* (2017) identified a novel *hda6* mutant allele (designated *shi5*) with a spontaneous defense response. The *shi5* mutant displays increased resistance to *Pst* DC3000 and constitutively activates several defense genes, including *PRs* and *WRKYs* (Wang *et al.*, 2017). It was found that HDA6 binds to the promoter of both genes (Wang *et al.*, 2017). Moreover, histone acetylation levels at the promoter of many defense genes are up-regulated in *shi5* mutants (Wang *et al.*, 2017). Thus, HDA6 plays a vital role in inhibiting the expression of defense genes via the regulation of histone acetylation levels.

In contrast to histone deacetylases, histone acetyltransferases (HATs or HACs) are well-known transcriptional coactivators that facilitate transcription through a diverse set of functions including the relaxing of histones by acetylation making DNA more accessible to transcription factors (Ogryzko *et al.*, 1996; Barlev *et al.*, 2001). A recent study showed that CBP/p300-family HATs HAC1 and HAC5 are essential in developing SA-triggered immunity and expression of genes involved in the SA pathway, such as *ICS1*, *EDS5*, *PAD4*, and *PR1* (Jin *et al.*, 2018). The *hac1-2* single and *hac1-2 hac5-2* double mutants are more susceptible to *Pst* DC3000 infection and accumulate significantly lower amounts of *PR1* transcript (Jin *et al.*, 2018). Both SA analog 2,6-dichloroisonicotinc acid (INA) and *Pst* DC3000 induce H3Ac levels in *PR1* chromatin in Col-0 but not in *hac1-2 hac5-2* double mutants, indicating that HAC1 and HAC5 are required for histone 3 acetylation in *PR1* chromatin (Jin *et al.*, 2018). Interestingly, HAC1 and HAC5 interact with NPR1 to form a coactivator complex with TGAs (Jin *et al.*, 2018). Thus, HAC1/5–NPR1–TGAs are recruited to the *PR* chromatin to activate *PR* transcription by histone acetylation-mediated epigenetic reprogramming (Fig. 2).

Dynamic histone methylation and demethylation play key roles in plant defense gene expression

Histone lysine methylation activates or represses transcription depending on the specific residue that is modified (Shilatifard, 2006). A recent study revealed that Arabidopsis histone methyltransferases SET DOMAIN GROUP8 (SDG8) and SDG25 regulate peptide-triggered immunity as well as SAR (Lee *et al.*, 2016). The *sdg8* and *sdg25* mutants are more susceptible to the bacterial pathogen *Pst* DC3000 with or without *avrB* and the fungal pathogen *Botrytis cinerea* (Lee *et al.*, 2016). The RNA-seq and qPCR data revealed that SDGs regulate a diverse set of immune response genes implicated in bacterial and fungal resistance, such as *PR1* and *PDF1.2* (Lee *et al.*, 2016). Loss of immunity in *sdg* mutants was attributed to altered global and *CAROTENOID ISOMERASE2* (*CCR2*)- and *ECERIFERUM3* (*CER3*)-specific histone lysine methylation (Lee *et al.*, 2016). Thus, SDG8 and SDG25 contribute to plant immunity directly through histone lysine methylation of plant immunity genes to regulate expression (Fig. 2).

Histone demethylases, which remove methyl groups from histone proteins, are also involved in SA-mediated immunity. A recent study identified a JmjC domain-containing histone demethylase 2 (JmijC domain-containing protein, JMJ27) that modulates defense against pathogens as well as flowering time (Dutta *et al.*, 2017). *JMJ27* is induced in response to virulent *Pseudomonas syringae* pathogens and is required for resistance against these pathogens (Dutta *et al.*, 2017). In *jmj27-1* mutants, the expression of *PR1*, *PR3*, *PR4*, and *PR5* genes is dramatically reduced but not *PR2* compared with wild-type plants challenged with *Pst* DC3000 (Dutta *et al.*, 2017). ChIP-qPCR results show that levels of H3K9me2 at the *PR1* promoter in *jmj27* mutants are increased compared with Col-0, indicating that loss of JMJ27 function leads to hypermethylation of histones at the *PR1* promoter (Fig. 2) (Dutta *et al.*, 2017).

DNA methylation: a new player in salicylic acid-mediated plant immunity

In plants, DNA methylation plays an important role in silencing transposable elements (TEs) and endogenous genes (Zhang, 2012). In addition, DNA methylation can also affect chromatin structure (Martinowich *et al.*, 2003). In Arabidopsis,

DNA is methylated at CG, CHG, and CHH sequences (where H is A, C, or T) through three genetically separate pathways (López *et al.*, 2011). Cytosine methylation is established by *de novo* methyltransferases (DRM1/2) through the RNA-directed DNA methylation (RdDM) pathway (Fig. 2) (Cao and Jacobsen, 2002). Methylation of CGs and CHGs is maintained through DNA replication by MET1 (cytosine methyltransferase), and the plant-specific Chromomethylase 3 (CMT3) methyltransferase, respectively (Lindroth *et al.*, 2001; Kankel *et al.*, 2003). The *met1* and *drm1 drm2 cmt3* (*ddc*) mutants are more resistant to the bacterial pathogen *Pst* DC3000 (López *et al.*, 2011; Matzke and Mosher, 2014). Subsequent RNA-seq data showed that many defense genes were constitutively expressed in *met1* and *ddc* mutants in the absence of infection, indicating that DNA methylation negatively regulates defense genes expression (Agorio and Vera, 2007). Genome-wide methylation profiling (MethylC-seq) revealed that DNA methylation dynamically responds to pathogen infection and SA treatment, further confirming the role of DNA methylation in SA-mediated plant immunity (Dowen *et al.*, 2012). DNA methylation is antagonistically controlled by DNA demethylases. Conversely, hyper-methylated *ros1* (*repressor of silencing 1*, encoding a DNA demethylase) mutants displayed reduced *PR1* gene expression (Fig. 2) (López Sánchez *et al.*, 2016). Together, these data indicate that DNA methylation plays negative roles in SA-mediated plant defense by down-regulating defense gene expression.

As an important component in the RdDM pathway, ARGONAUTE 4 (AGO4) plays a vital role in SA-mediated plant immunity (Fig. 2). *ago4* mutants are susceptible to *Pst* DC3000 and *Pst* DC3000 (*avrRPM1*) (Agorio and Vera, 2007). The extent of DNA cytosine methylation at CpNpG and CpHpH positions of *Ep5C* (which encodes an extracellular peroxidase in tomato) promoter region was reduced, leading to the constitutive expression of *Ep5C*, a negative regulator of defense, in *ago4* mutants (Agorio and Vera, 2007). RNA Polymerase V is another crucial component in the RdDM pathway (Fig. 2). NRPE1 and NRPD2 encode the largest and second-largest subunits of the Pol V complex, respectively (Fig. 2) (López *et al.*, 2011). Interestingly, both *nrpe1* and *nrpd2* mutants show enhanced disease resistance towards *Pst* (López *et al.*, 2011), but it is not clear whether this is a direct or indirect effect on disease-related genes (López *et al.*, 2011). AGO4 and Pol V function differently in SA-mediated defense in the RdDM pathway (Fig. 2). A deeper understanding of how RdDM regulates SA-mediated plant immunity warrants further research.

Small RNAs fine-tune salicylic acid-mediated plant immunity

Besides histone modifications and DNA methylation, small RNAs play a crucial role in the epigenetic regulation of plant immunity (Weiberg *et al.*, 2014). For instance, Arabidopsis *miR393* (*microRNA393*) induced by flg22 contributes to resistance against *Pst* DC3000 by down-regulating auxin-responsive genes, which antagonize SA and SA signaling (Fig. 2) (Navarro *et al.*, 2006). *miR393**, a complementary strand of *miR393*, is loaded into AGO2 and regulates plant immunity by suppressing the *MEMBRIN 12* (*MEMB12*) gene, which causes the increased exocytosis of antimicrobial PR proteins (Fig. 2) (Zhang *et al.*, 2011). The first small interfering RNA (siRNA) reported to regulate plant immunity was nat-siRNAATGB2, which is highly and specifically induced by *Pst avrRpt2* (Fig. 2) (Katiyar-Agarwal *et al.*, 2006). RPS2- and NDR1-dependent induction of nat-siRNAATGB2 leads to the silencing of the antisense gene *Pentatricopeptide repeats protein-like* (*PPRL*), a negative regulator of the RPS2 resistance pathway (Fig. 2) (Katiyar-Agarwal *et al.*, 2006). In response, *Pseudomonas syringae* developed strategies to suppress transcriptional activation of PAMP-responsive miRNAs (microRNAs) (Navarro *et al.*, 2008). For instance, AvrPtoB down-regulates pri-miR393a and pri-miR393b accumulation independently of its E3-ligase activity (Fig. 2) (Navarro *et al.*, 2008). In addition, AvrPto and HopT1-1 suppress the accumulation and stability of miRNAs (Navarro *et al.*, 2008).

Concluding remarks and future directions

Significant progress has been made in understanding the biosynthesis and signaling of SA in plant immunity over the past two decades. However, the SA biosynthesis pathway has yet to be fully elucidated. For example, in the PAL-mediated SA biosynthesis pathway, the gene that encodes benzoic acid 2-hydroxylase, the last step in catalysing benzoic acid to SA, has not been identified in Arabidopsis (Dempsey *et al.*, 2011). Similarly, it has been implicated that EDS5 functions as a transporter of SA, which was postulated from evidence that SA was trapped in the chloroplast of *eds5* mutants (Serrano *et al.*, 2013), but a recent study indicates that EDS5 functions to transport isochorismate rather than SA (Rekhter *et al.*, 2019). A more recent paper provided evidence that EDS5 is required for UV-C induced pipecolic acid production likely by exporting pipecolic acid from the chloroplast to the cytosol (Rekhter *et al.*, 2019). Further studies on the exact function of EDS5 will help us better understand how SA is synthesized.

The transcription of SA biosynthesis gene regulators has been extensively studied and significant progress in this area has been made. Several transcription factors that regulate *ICS1*, *EDS1*, *PAD4*, *PBS3*, *EDS5*, and *EPS1* have been identified. However, a full understanding of the transcription network remains elusive, especially the transcription of *EPS1*, *PBS3*, and *PAD4*, which until recently has been poorly understood. Moreover, the enzymatic activity of EDS1 and PAD4 remains elusive. Although EDS1 and PAD4 possess potential lipase activity, this activity has not been demonstrated so far. To fully uncover the transcription network underlying the SA signaling pathway, next-generation technologies, such as DNase-seq, MNase-seq, FAIRE-seq, and ATAC-seq, must be utilized. These advanced techniques represent an excellent opportunity for identifying regulatory sequences in their native chromatin environment on a genome-wide scale. The adoption of CRISPR/Cas9 for genomically editing epitope-tags onto TFs in combination with ChIP (CETCh-seq) will continue to

facilitate and increase our understanding of the defense transcription network (Birkenbihl *et al.*, 2017). In addition, Hi-C, as a genome-wide sequencing technique, can be used to investigate 3D chromatin conformation inside the nucleus (Forcato *et al.*, 2017).

Small RNAs play a crucial role in epigenetic regulation of plant immunity (Weiberg *et al.*, 2014). However, it is not clear if miRNA(s) produced by pathogens can specifically target the SA signaling pathway. Deep RNA sequencing could be useful to identify novel siRNAs and miRNAs produced by pathogens suppressing SA-mediated plant immunity, and vice versa, the identification of plant siRNAs and miRNAs that regulate SA-mediated plant defense could further expand our knowledge and understanding of plant-pathogen interactions.

Acknowledgements

This work was supported by the National Science Foundation (EAGER grant 1464527 and grant IOS-1758994 to ZF). JC is supported by a JAAS postdoc fellowship.

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