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REVIEW

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More stories to tell: NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1, a salicylic acid receptor

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Abstract

Salicylic acid (SA) plays pivotal role in plant defense against biotrophic and hemibiotrophic pathogens. Tremendous progress has been made in the field of SA biosynthesis and SA signaling pathways over the past three decades. Among the key immune players in SA signaling pathway, NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1 (NPR1) functions as a master regulator of SA-mediated plant defense. The function of NPR1 as an SA receptor has been controversial; however, after years of arguments among several laboratories, NPR1 has finally been proven as one of the SA receptors. The function of NPR1 is strictly regulated via post-translational modifications and transcriptional regulation that were recently found. More recent advances in NPR1 biology, including novel functions of NPR1 and the structure of SA receptor proteins, have brought this field forward immensely. Therefore, based on these recent discoveries, this review acts to provide a full picture of how NPR1 functions in plant immunity and how *NPR1* gene and NPR1 protein are regulated at multiple levels. Finally, we also discuss potential challenges in future studies of SA signaling pathway.

KEYWORDS
Arabidopsis thaliana, NPR1, plant immunity, post-translational modification, regulatory network, transcription regulation

1 | INTRODUCTION

In the natural environment, plants are constantly facing the challenge of various kinds of pathogenic infection, such as fungi, oomycetes, viruses, bacteria and nematodes (Chen et al., 2020). Plant pathogens

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cause severe loss in terms of economics and production in the agricultural sector. Worldwide, yield losses caused by diseases are estimated to average 10 to 40% (Savary et al., 2019). To deal with these challenges, plants have developed a sophisticated and multifaceted immune system to combat pathogens (Sun et al., 2020).

The first line of defense is the physical barriers to infection, such as the cuticle and the cell wall. Pathogens that are able to overcome these physical barriers encounter an evolved, multilayer system of immune responses, including both innate and acquired responses (Jones & Dangl, 2006). The plant's innate immune response depends on two main recognition systems to detect invaders. One system is initiated by the recognition of pathogen/microbe-associated molecular patterns (PAMPs or MAMPs) via pattern recognition receptors (PRRs), leading to PAMP-triggered immunity (PTI) (Dodds & Rathjen, 2010). One typical elicitor of PTI is the bacterial flagellin, which triggers defense responses in various plants (Gomez-Gomez & Boller, 2002). Flg22, a 22-amino acid sequence of the conserved N-terminal part of flagellin, is sufficient to induce full defense in plants. Flg22 is recognized by the receptor-like kinase FLAGELLIN SENSITIVE 2 (FLS2), which acts together with another receptor-like kinase, BRASSINOSTEROID INSENSITIVE1-associated receptor kinase 1 (BAK1), to activate downstream immune responses (Chinchilla et al., 2007; Chinchilla, Bauer, Regenass, Boller, & Felix, 2006; Zipfel et al., 2004).

To suppress PTI, pathogens have evolved a set of proteins called effectors to suppress the immune system of the plants. In this competition, plants have also evolved to encompass a second layer of their immune system. The second layer involves intracellular host receptors encoded by major resistance genes to detect pathogen-derived effector molecules within the host cell, resulting in effector-triggered immunity (ETI). ETI is qualitatively stronger than PTI and culminates in hypersensitive response. For instance, bacterial effectors from *Pseudomonas syringae*, AvrRpm1 and AvrB, are recognized by RESISTANCE TO PSEUDOMONAS SYRINGAE PV. MACULICOLA 1 (RPM1) protein in *Arabidopsis thaliana* results in enhanced defense responses, cessation of pathogen growth and hypersensitive host cell death at the infection site (Desveaux et al., 2007; Mackey, Holt 3rd, Wiig, & Dangl, 2002). Another well-known bacterial effector AvrRpt2 is recognized by the resistance protein RESISTANT TO PSEUDOMONAS SYRINGAE2 (RPS2) in *A. thaliana* to trigger ETI (Kunkel, Bent, Dahlbeck, Innes, & Staskawicz, 1993). Most resistance genes encode nucleotide-binding leucine-rich repeat (NLR) proteins (Collier & Moffett, 2009). In general, most plants carry a repertoire of 50–1,500 different *NLR* genes that mediate resistance to various viruses, bacteria, fungi, oomycetes and nematodes (van Wersch, Tian, Hoy, & Li, 2020).

Besides the induction of defense at the infection site, a common feature of both PTI and ETI is the activation of systemic defense response, which is often triggered in the distal parts of the infected plants. The activation thereby protects uninfected tissues against subsequent infections by a wide range of pathogens. The long-lasting- and broad-spectrum-induced disease resistance previously described is referred to as systemic acquired resistance (SAR) (Pieterse, Leon-Reyes, Van der Ent, & Van Wees, 2009). SAR is associated with increased levels of plant hormone salicylic acid (SA), a beta-hydroxy phenolic acid, at the site of infection and in systemic tissues. SA is widely produced in prokaryotes and plants. Accumulation of SA leads to the induction of *pathogenesis-related (PR)* genes, some of which encode proteins with antimicrobial activity (van Loon, Rep, & Pieterse, 2006).

Over the last three decades, significant progress has been made in deciphering plant immune signaling governed by plant hormone SA. This review summarizes our current understanding concerning the function of SA and one of its receptors, NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1 (NPR1), in plant immunity (Figure 1). In addition, we highlight recent breakthroughs in the perception of SA because of its critical roles in the potentiation of PRRs- and NLRs-mediated signaling (Figure 1). Finally, we focus on recent breakthroughs that have substantially advanced our understanding of how NPR1 is regulated at different levels.

2 | ROLES OF SA AND ONE OF ITS RECEPTORS NPR1 IN PLANT IMMUNITY

SA is a phytohormone that plays a vital role in plant defense against biotrophic and semi-biotrophic pathogens (Fu & Dong, 2013; Qi et al., 2018). The first observation of SA's involvement in plant immunity was reported by Raymond F. White in 1979. He discovered that the application of aspirin in tobacco conferred resistance against tobacco mosaic virus (TMV) (White, 1979). Studies also found that SA levels increased in phloem sap collected from cucumber and tobacco leaves inoculated with SAR-inducing pathogens (Malamy, Carr, Klessig, & Raskin, 1990; Métraux et al., 1990). These pioneer works suggested that endogenous SA plays a role as an internal defense signal for plant immunity.

2.1 | Transportation and function of SA in SAR

One of the best characterized roles for SA in plant immunity is its role in SAR (Gaffney et al., 1993). SA was initially considered as a mobile signal for SAR because the concentration of SA increases in both the primary infected and systemic uninfected tissue (Malamy et al., 1990). Grafting experiments suggested that methyl salicylate (MeSA) is a critical, phloem-mobile SAR long-distance signal in tobacco (Park, Kaimoyo, Kumar, Mosher, & Klessig, 2007); however, the subsequent study concluded that MeSA is not the mobile signal for SAR (Attaran, Zeier, Griebel, & Zeier, 2009). Later, it was confirmed that SA is a mobile signal because the pathogen-induced SA could move via the apoplast compartment (Lim et al., 2016). Indeed, it was shown that the SA level was increased in the apoplast collected from *P. syringae* pv. *tomato* (*Pst*) DC3000 carrying *avrRpt2* inoculated plants when compared with that in mock-inoculated plants (Lim et al., 2016). In contrast, two other SAR-associated chemical signals, glycerol-3-phosphate (G3P) and azelaic acid (AzA), are transported

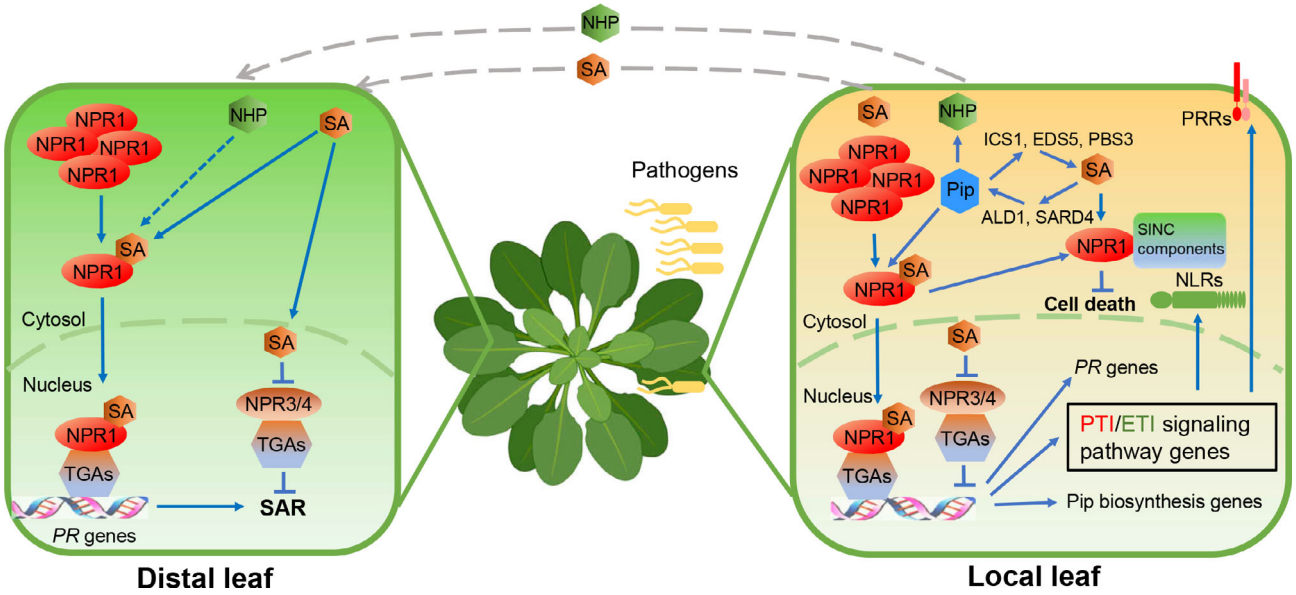

FIGURE 1 Schematic overview of the function of SA and NPR1 in plant immunity. Upon infection of pathogens, SA is perceived by its receptors. Binding of SA abolishes the transcriptional repression activity of NPR3/4 and activate the transcriptional activation activity of NPR1. Cytoplastic NPR1 forms condensates to repress cell death. Nuclear localized NPR1 upregulates SA-responsive defense regulators such as *PR* genes, genes encoding PTI/ETI signaling components and pipecolic acid biosynthetic genes. SA and Pip form an amplification loop by activating Pip or SA biosynthetic genes (*ICS1*, *EDS5*, *PBS3*, *ALD1* and *SARD4*) to promote SA and NHP production. NHP and SA may act as SAR mobile signals to further activate the expression of SA biosynthetic genes thereby promote SA biosynthesis and SA-induced SAR [Colour figure can be viewed at wileyonlinelibrary.com]

preferentially via plasmodesmata (Lim et al., 2016). More interestingly, Lim et al. (2020) recently showed that transport of SA from local to distal tissues is essential for SAR, and the transportation is governed by water potential in the infected tissue (Figure 1). Indeed, reduced water potential preferentially routes SA to cuticle wax rather than to the apoplast in cuticle-defective mutants (Lim et al., 2020).

N-hydroxyl pipecolic acid (NHP), which is catalyzed from pipecolic acid (Pip) by flavin-containing monooxygenases 1 (FMO1), was recently suggested as a mobile signal for SAR (Y. C. Chen et al., 2018; Hartmann et al., 2018). SA contributes to the induction of Pip biosynthesis (Figure 1). Pip biosynthetic genes *AGD2-LIKE DEFENSE RESPONSE PROTEIN 1* (*ALD1*) and *SYSTEMIC ACQUIRED RESISTANCE DEFICIENT 4* (*SARD4*) are upregulated upon SA treatment (Ding et al., 2018). In the *npr1-1* mutant, the accumulation of Pip in the primarily infected leaves is delayed, suggesting that NPR1 positively regulates Pip biosynthesis (Návarová, Bernsdorff, Döring, & Zeier, 2012). Interestingly, SA biosynthetic genes *ISOCHORISMATE SYNTHASE 1* (*ICS1*), *ENHANCED DISEASE SUSCEPTIBILITY 5* (*EDS5*) and *AVRPPHB SUSCEPTIBLE 3* (*PBS3*) are induced by Pip (Figure 1) (Hartmann et al., 2018). Moreover, Pip induces NPR1 protein level without induction of *NPR1* expression or increase of SA levels (Figure 1) (Y. Kim, Gilmour, Chao, Park, & Thomashow, 2020). In all, the data suggests that there are amplification loops between SA and Pip signaling. Similarly, a recent finding suggested that SA and NHP pathways can mutually amplify each other (Sun et al., 2020). The mutual amplification suggests that the SA and NHP pathways are coordinated to optimize plant immune response (Figure 1).

2.2 | SA amplifies PTI signal

SA also plays a critical role in PTI. Resistance against *Pst* DC3000 induced by PTI elicitors flg22 and elf18 was compromised in SA biosynthesis mutant *sid2-2* (Tsuda, Sato, Stoddard, Glazebrook, & Katagiri, 2009). In addition, *sid2-2* and *npr4-4D* (carries a gain-of-function mutation in SA receptor encoding gene *NPR4*, which constitutively represses SA signal) mutants are more susceptible to *Pst* DC3000 *hrcC*$^{-}$, a type-III secretion system-deficient bacterial strain (Ding et al., 2018; Tsuda, Sato, Glazebrook, Cohen, & Katagiri, 2008). In agreement with the SA biosynthesis pathway, a recent study showed that the SA receptor NPR1 plays a prominent role in MAMP signaling (H. Chen et al., 2017). NPR1 positively regulates cell wall-associated plant defense in response to the *Pst* DC3000 *hrcC*$^{-}$ (H. Chen et al., 2017). Activation of the early MAMP marker genes was also significantly impaired in *npr1-2* mutant after pathogen challenge (H. Chen et al., 2017). SA rapidly induces genes encoding PAMP receptors such as *FLS2*, *EF-Tu receptor* (*EFR*), *CHITIN ELICITOR RECEPTOR KINASE 1* (*CERK1*) and co-receptor *BAK1-LIKE 1* (*BKK1*) (Figure 1) (Ding et al., 2018; Tateda et al., 2014). In addition, SA also induces a large number of genes encoding signaling components that act downstream of PAMP receptors, such as constituents of mitogen-activated protein (MAP) kinase cascades, including *MAPKKK5*, *MKK1*, *MKK2*, *MKK4* and *MPK11*. The same holds true for the subunits of heterotrimeric G proteins, such as *EXTRA-LARGE G-PROTEIN 2* (*XLG2*) and *ARABIDOPSIS G-PROTEIN GAMMA-SUBUNIT 1* (*AGG1*) (Ding et al., 2018; Y. Zhang & Li, 2019). Thus, SA biosynthesis and NPR1-mediated SA signaling play prominent roles in PTI process.

2.3 | SA plays a dual role in ETI

SA plays dual role in ETI. To begin, SA is required for ETI. Initial evidence came from the finding that *A. thaliana* expressing the bacterial enzyme salicylate hydroxylase cannot accumulate SA and is, therefore, more susceptible to the ETI elicitor *Pst avrRpt2* (Delaney et al., 1994). Early studies revealed that SA accumulation is associated with the onset of a hypersensitive response during resistance gene-mediated defense responses (Nawrath & Metraux, 1999). Activation of ETI by *Pseudomonas* effectors AvrRpm1 and AvrRpt2 in Arabidopsis results in dramatic increases in local SA levels, which occurs in an ICS1- and EDS5-dependent manner (Nawrath & Metraux, 1999). SA amplifies ETI signal through positive regulation of several sensor NLR genes, such as *RPM1*, *RPS6*, *HOPZ-ACTIVATED RESISTANCE 1* (*ZAR1*) and *RESISTANCE TO LEPTOSPHAERIA MACULANS 3* (*RLM3*) (Figure 1) (Ding et al., 2018). Interestingly, *ENHANCED DISEASE SUSCEPTIBILITY1* (*EDS1*), *PHYTOALEXIN DEFICIENT4* (*PAD4*), *SENESCENCE-ASSOCIATED GENE 101* (*SAG101*) and *NON RACE-SPECIFIC DISEASE RESISTANCE 1* (*NDR1*), which are required for TNL (Toll-like/Interleukin 1 receptor-type NLR)- and CNL (coiled-coil-type NLR)-mediated plant immunity, are also induced by SA (Ding et al., 2018; Falk et al., 1999; Feys, Moisan, Newman, & Parker, 2001).

On the other hand, the activation of SA signaling also plays an essential role in the negative regulation of cell death during ETI. It was shown that SA pre-treatment in Arabidopsis Col-0 plants blocks HR activated by *P. syringae* pv. *maculicola* ES4326 carrying *avrRpm1* (Devadas & Raina, 2002). In addition, increased cell death was observed in *eds5-3* compared to wild type infected with *Pst* DC3000 *avrRpt2* (Radojicic, Li, & Zhang, 2018). Finally, it was revealed that SA-mediated suppression of cell death is dependent on NPR1. The evidence in support of this finding is that *npr1* mutants show a stronger HR, while NPR1-overexpressing plants show a weaker HR when

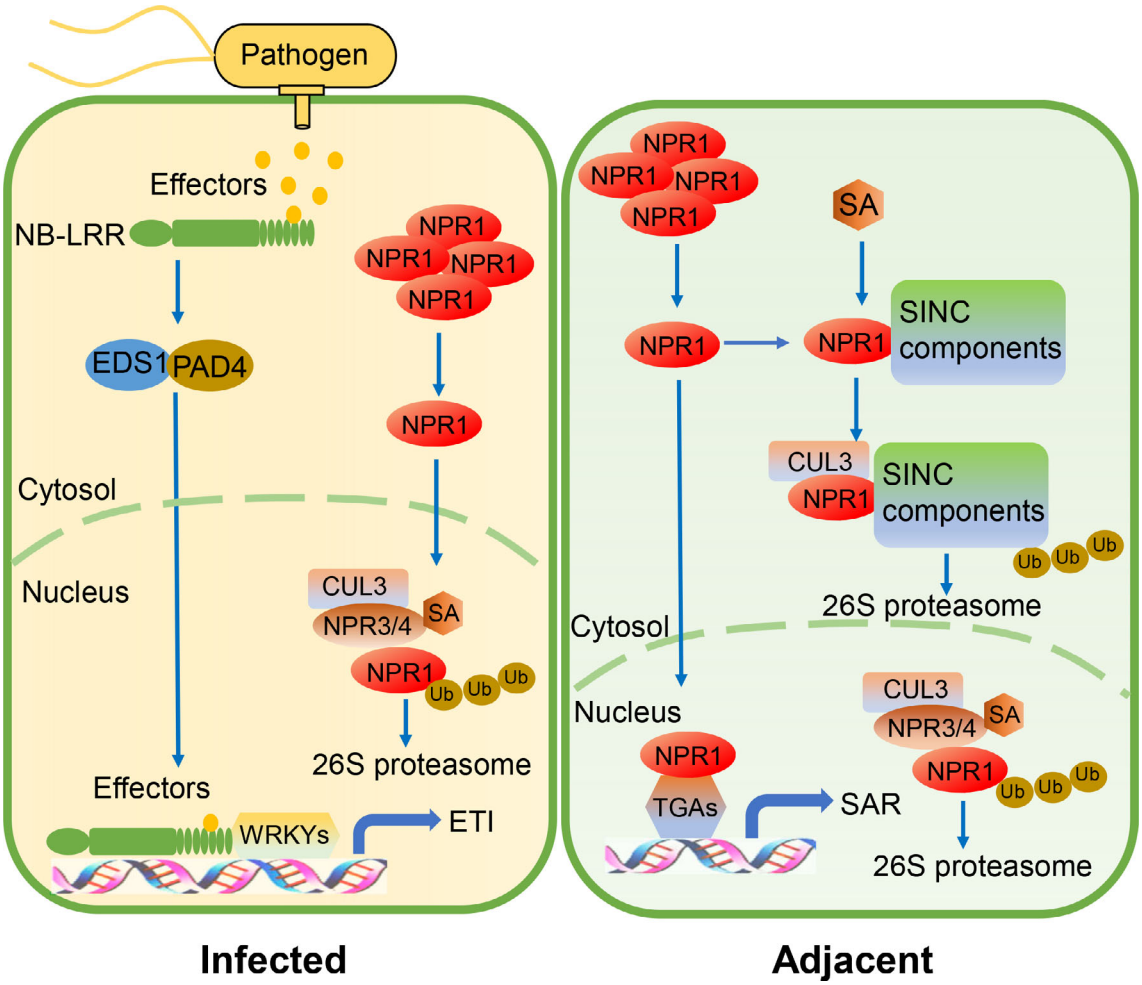

FIGURE 2 NPR1 promotes survival during ETI. During ETI, infected cells detect pathogen effectors through nucleotide-binding leucine-rich repeat (NB-LRR) proteins. The signal is then transduced through components such as EDS1/PAD4 and WRKY transcription factors. NPR1 monomers enter the nucleus and are subjected to the NPR3/4-CUL3 complex for degradation to remove its inhibitory effect on ETI. In adjacent cells, SA mediates the activation of nuclear NPR1 to induce transcription of SAR genes, including salicylic acid-induced NPR1 condensates (SINC) components, such as NB-LRRs, EDS1 and WRKY54/70. In the presence of SA, NPR1 promotes cell survival by recruiting CUL3 and targeting substrates that are involved in cell death for ubiquitination and degradation through the formation of SINCs [Colour figure can be viewed at wileyonlinelibrary.com]

infected with *P. syringae* carrying the *avrRpm1* gene (Rate & Greenberg, 2001).

Very recently, a breakthrough study unveiled a detailed mechanism by which NPR1 promotes defense and restricts cell death (Figure 2) (Zavaliev, Mohan, Chen, & Dong, 2020). Zavaliev et al. (2020) showed that NPR1 promotes cell survival by targeting substrates for ubiquitination and degradation through the formation of SA-induced NPR1 condensates (SINCs) in cytoplasm (Figure 2). Upon SA induction, NPR1 interestingly assembles many proteins into punctate structures to form the SINCs in cytoplasm. Within the SINC, NPR1 assembles into a CUL3-based E3 ubiquitin ligase complex to ubiquitinate SINC-localized substrates, such as EDS1 and specific WRKY transcription factors, and promote cell survival during ETI (Figure 2) (Zavaliev et al., 2020). It is worthwhile to note that NPR1 recruits EDS1 into SINCs through direct interaction. Because EDS1 is a major upstream immune regulator involved in ETI, NPR1/EDS1 protein complex may serve as a master regulator in transcriptional reprogramming and cell death. It is still a mystery how exactly SINCs are formed. Zavaliev et al. (2020) hypothesize that SINCs form via liquid–liquid phase separation (LLPS), but this is not fully demonstrated. Redox-sensitive disordered regions (RDRs) can be inhibitory to phase separation. For this reason, Zavaliev et al. (2020) identified and tested the role of several disordered regions in NPR1 condensate assembly. They found that RDR1 and RDR2 are inhibitory to NPR1 condensate assembly, and RDR3 drives NPR1 condensate assembly (Zavaliev et al., 2020). Despite these discoveries, understanding how SA-induced SINC assembly is initiated still requires additional mechanistic studies.

3 | NPR1, NPR3 AND NPR4 ARE *BONA FIDE* SA RECEPTORS

3.1 | SA perception by NPR1/3/4

It is widely believed that plant and animal hormone molecules transduce their signals by binding to one or more receptors. Despite the discovery of dozens of SA binding proteins (SABPs) (Z. Chen, Ricigliano, & Klessig, 1993; Ding et al., 2018; Du & Klessig, 1997; Fu et al., 2012; Kumar & Klessig, 2003; Manohar et al., 2015; Slaymaker et al., 2002; Yuan, Liu, & Lu, 2017), only NPR1, NPR3 and NPR4 are proved to be the bona fide SA receptors that function as transcriptional regulators (Ding et al., 2018; Fu et al., 2012; Wu et al., 2012). NPR3 and NPR4 display a high affinity with SA, while the SA binding activity of NPR1 was controversial (Ding et al., 2018; Fu et al., 2012; Wu et al., 2012).

Until very recently, W. Wang et al. (2020) re-evaluated SA binding by NPR1 and found that less than 0.02% of the total MBP–NPR1 in the sample was able to bind SA, while about 8% of NPR4 were able to bind to SA at the same saturating concentration. The findings of this observation explain why the SA binding activity of NPR1 was barely detected, which is reported by Fu et al. (2012). Furthermore, W. Wang et al. (2020) identified amino acids 373–516 within the NPR4 C-terminal domain as the SA-binding core (SBC). W. Wang et al. (2020) also characterized SBC surface residues that affect SA binding and found that three mutants, NPR4(F426L), NPR4(E469I) and NPR4(K505Q), showed reduced SA binding activity. By contrast, mutations of T459 to G increased SA binding to NPR4 by 50% (W. Wang et al., 2020). Interestingly, when the T459G substitution was combined with F426L, the ability of NPR4 to bind SA was substantially enhanced. The identification of important amino acid residues of NPR4 proteins could be helpful for a new direction for engineering plant immunity. NPR1 and NPR4 share nearly identical hormone-binding residues. NPR1 is also equipped with a potential SBC module (amino acids 386–525) that is capable of sensing SA (W. Wang et al., 2020). Despite NPR1 and NPR4 sharing nearly identical hormone-binding residues, NPR1 displays minimal SA-binding activity compared to NPR4.

3.2 | Structural basis of NPR proteins

Despite extensive efforts, the structure of NPR proteins has not been resolved until very recently. W. Wang et al. (2020) identified amino acids 373–516 within the NPR4 C-terminal domain as the SBC. Using hydrogen–deuterium-exchange mass spectrometry (HDX-MS), W. Wang et al. (2020) confirmed that the SBC of NPR4 has a deuterium uptake profile that is sensitive to. SBC of NPR4 was then crystallized and determined its structure at 2.3 Å resolution (W. Wang et al., 2020). The structure of the NPR4 SBC consists of five tightly packed α-helices and the C-terminal four-helix-bundle-like fold, while the SA-binding site is located at the tapered end of the four-helix bundle of the SBC of NPR4 (W. Wang et al., 2020). The SA-binding pocket is characterized by its hydrophobicity and its central location within the receptor SBC domain. The pocket completely buries the SA inside an internal cavity at the tapered end of the four-helix-bundle-like fold, leaving no gap for the ligand to enter or escape (W. Wang et al., 2020). The lack of a ligand-entry pathway suggests that SA binding involves a major conformational remodeling of the NPR4 SBC (W. Wang et al., 2020). By revealing the structural mechanisms of SA perception by NPR4 SBC, Wang et al. provide initial insights into the structure–function relationships of NPR proteins, which in turn sheds light on the interplay between NPR proteins in SA signaling and provides a new direction for engineering plant immunity.

3.3 | SA signaling by NPR1/3/4

As an SA receptor, NPR1 functions as a transcriptional activator that promotes SA-induced defense gene expression. The *npr1* mutant was first identified during a screening of Arabidopsis mutants that do not respond to SA or its active analogs (Cao, Bowling, Gordon, & Dong, 1994; Delaney, Friedrich, & Ryals, 1995). Loss of NPR1 results in reduced *PR* gene expression and increased susceptibility to pathogens (Cao et al., 1994; Delaney et al., 1995). NPR1 consists of an N-terminal bric-a-brac, tramtrack, and broad-complex (BTB) domain,

ankyrin repeats and a C-terminal domain containing a nuclear localization signal (NLS) and a putative transactivation domain (Kinkema, Fan, & Dong, 2000; Rochon, Boyle, Wignes, Fobert, & Despres, 2006). The NLS is required for SA-induced NPR1 nuclear translocation and function in SAR (Kinkema et al., 2000). 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 it is translocated to the nucleus to activate downstream transcription cascades (Mou, Fan, & Dong, 2003).

NPR1 itself does not have a DNA binding domain, so NPR1-mediated signaling requires interaction with other transcription factors. Yeast two-hybrid screening has revealed that NPR1 interacts with seven members of the TGA transcription factor family (Boyle et al., 2009; Despres, DeLong, Glaze, Liu, & Fobert, 2000; H. S. Kim & Delaney, 2002; Zhou et al., 2000). Apart from transcription factors, epigenetic regulators are also crucial for the transcription. Histone acetyltransferases (HATs or HACs) are well-known transcriptional coactivators that facilitate transcription through relaxing specific chromatin regions by histone acetylation, which makes DNA more accessible to transcription factors (Barlev et al., 2001; Ogryzko, Schiltz, Russanova, Howard, & Nakatani, 1996). Collectively, HAC1 and HAC5 interact with NPR1 to form a coactivator complex with TGAs recruited to the *PR* chromatin to activate the transcription of *PR* genes (Jin et al., 2018).

NPR1 positively regulates SA-mediated plant immunity, whereas the major functions of NPR3 and NPR4 are as negative regulators of plant defense (Figure 1) (Fu et al., 2012; Y. Zhang et al., 2006). Previously, it was proposed that NPR3 and NPR4 function as adaptors of the Cullin3 ubiquitin 3 E3 ligase (CUL3) to mediate NPR1 degradation (Fu et al., 2012); however, Ding et al. (2018) did not recognize the function of NPR3 and NPR4 as Cullin3 E3 ligases. To solve this discrepancy, sophisticated biochemical studies are required to see whether or not NPR3 and NPR4 function as adaptors to degrade NPR1 in vitro. Nevertheless, the *npr3 npr4* double mutants do accumulate higher levels of NPR1 protein (Fu et al., 2012). Consistent with this, W. Wang et al. (2020) demonstrate that NPR1 is destabilized in NPR4 (F426L/T459G) and NPR4 transgenic plants when treated with 1 mM SA. In addition, NPR3 and NPR4 have been shown to facilitate the degradation of JASMONATE ZIM DOMAIN (JAZ) proteins to promote ETI (L. Liu et al., 2016).

In addition to regulating NPR1 protein level, NPR3 and NPR4 serve as redundant transcriptional co-repressors that prevent activation of defense gene expression when SA levels are low. NPR3/NPR4 negatively regulates the expression of *SARD1*, *CBP60g* and *WRKY70* through interaction with transcription factors TGA2/TGA5/TGA6 (Ding et al., 2018). In contrast, NPR1 promotes the expression of *SARD1* and *WRKY70* in response to SA (Ding et al., 2018). In the presence of SA, the transcription repressor activity of NPR3/4 was inhibited (Ding et al., 2018). The SA insensitive *npr4-4D* mutant not only displays enhanced disease susceptibility but also blocks INA-induced disease resistance (Ding et al., 2018). Thus, NPR1 and NPR3/4 play opposite roles in transcriptional regulation of SA-induced gene expression, although both are considered as bona fide SA receptors.

4 | TRANSCRIPTIONAL REGULATION OF *NPR1* GENE

Despite its pivotal role in plant immunity, the transcriptional regulation of *NPR1* is not extensively studied. As of now, there are only two transcription factors that have been found to bind to *NPR1* promoter (Chai, Liu, Zhou, & Xing, 2014; Yu, Chen, & Chen, 2001). WRKY18 was the first transcription factor that was reported to recognize the W-box motif directly and specifically in the *NPR1* promoter (Yu et al., 2001). The W-box motif in the *NPR1* promoter is essential for its gene expression (Yu et al., 2001). Mutations in the W-box sequences abolish their recognition by WRKY DNA binding proteins, rendering the promoter unable to activate a downstream reporter gene (Yu et al., 2001). The *npr1* mutants containing an *NPR1* gene with a mutated W-box in its promoter are unable to induce SA-dependent gene expression or pathogen resistance (Yu et al., 2001). SA induces a number of other *WRKY* genes suggesting that additional WRKY family proteins are involved in the regulation of *NPR1* gene expression. Indeed, ChIP assay showed that WRKY6 binds to the W-box of the *NPR1* promoter (Chai et al., 2014). Further analyses showed that the mRNA level of *NPR1* is reduced in *wrky6* mutants and enhanced in *WRKY6* overexpressing lines. *WRKY6*-induced *NPR1* gene expression is required for SA-induced leaf senescence, but it is not clear if *WRKY6* is involved in SA-mediated plant immunity.

Interestingly, NPR1 protein also regulates its own gene expression indirectly by recruiting CDK8 and WRKY transcription factors. The long-found understanding was that *NPR1* transcript accumulation in the *npr1* mutants was not induced by INA (Kinkema et al., 2000). Later, Zhang et al. (2012) showed that *Pst* DC3000-induced *NPR1* transcript accumulation in *npr1-3* mutant was significantly lower than that in wild type. The previously mentioned studies indicate that a functional NPR1 protein is required for the full expression of *NPR1*. Recently, we demonstrated that a functional NPR1 protein promotes *NPR1* gene expression by binding to its promoter (J. Chen et al., 2019). The finding that functional NPR1-GFP, but not npr1-2, is able to induce *npr1-2* gene expression demonstrates that NPR1 protein promotes its own gene expression. Since 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 (J. Chen et al., 2019). Despite these discoveries, there remain several gaps in our understanding of how *NPR1* expression is regulated. Finally, CYCLIN-DEPENDENT KINASE 8 (CDK8) filled this gap in knowledge (J. Chen et al., 2019) (Figure 3). NPR1 interacts with CDK8, which recruits RNA polymerase II to the promoter of *NPR1* to facilitate its gene expression (J. Chen et al., 2019). Interestingly, CDK8 also interacts with WRKY18 and WRKY6, which are positive regulators of *NPR1* (J. Chen et al., 2019). The investigation into how CDK8 regulates the transcription factor activity will be interesting. The investigation will give us a further understanding of how *NPR1* gene is regulated. CDK8 kinase module also includes Mediator 12 and 13 (MED12/13), mutation of which causes the plants to accumulate a low amount of *NPR1* transcript, which results in a loss of SAR (J. Chen

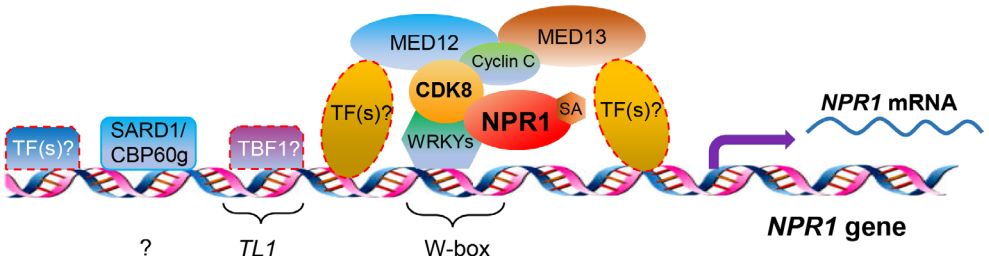

FIGURE 3 Transcriptional regulation of *NPR1* gene. Upon pathogen infection, SA accumulates in the plant cell. SA binds to NPR1 protein and promotes the interaction between NPR1 and WRKY18. CDK8 also interacts with WRKY transcription factors WRKY18 and WRKY6, which associate with *NPR1* promoter through W-box motif. In the presence of SA, NPR1 recruits CDK8 to *NPR1* promoter to facilitate its own gene expression. CDK8 kinase module subunits MED12 and MED13 are also involved in the transcriptional regulation of *NPR1* gene. Some unknown transcription factors that interact with MED12 or MED13 may regulate the expression of *NPR1* gene. TBF1 potentially regulates the expression of *NPR1* gene through the *TL1* element. SARD1 and CBP60g associate with *NPR1* promoter to regulate its gene expression through an unknown *cis*-element. Other unidentified transcription factors that regulate *NPR1* gene expression need to be discovered. TF, transcription factor [Colour figure can be viewed at wileyonlinelibrary.com]

et al., 2019). Further study is required to fully understand the role of MED12 and MED13 in the regulation of *NPR1* expression.

5 | POST-TRANSLATIONAL REGULATION OF NPR1 PROTEIN

5.1 | Conformational changes of NPR1

SA affects NPR1 function in two stages: first, it induces *NPR1* gene expression and second, it promotes the conformational change of NPR1 and facilitates the translocation of NPR1 into the nucleus. The function of NPR1 is tightly regulated by its conformational change. In an uninduced state, NPR1 is present as an oligomer formed through intermolecular disulfide bonds (Mou et al., 2003). There are 17 cysteine residues in NPR1, 10 of which are highly conserved from different plant species (Mou et al., 2003). Site-directed mutagenesis showed that this oligomer contains intermolecular disulfide bonds between cysteine residues positioned within the BTB domain (Cys^{82}) and the region in and adjacent to the BTB domain of NPR1 (Cys^{150}, Cys^{155}, Cys^{156}, Cys^{160} and Cys^{216}) (Mou et al., 2003). Mutations at residues Cys^{82} and Cys^{216} in NPR1 result in increased monomer accumulation, constitutive nuclear localization and NPR1-mediated gene expression in the absence of pathogen challenge (Mou et al., 2003). NPR1 is sensitive to redox changes. Upon SAR induction, a biphasic change in cellular reduction potential occurs, resulting in a reduction of NPR1 from oligomeric form to monomeric form (Mou et al., 2003). Monomeric NPR1 accumulates in the nucleus and activates defense gene expression. NPR1 conformational changes are regulated by S-nitrosylation and thioredoxins (Figure 4). S-nitrosylation of Cys^{156} by S-nitrosoglutathione (GSNO) facilitates the assembly of NPR1 oligomer (Tada et al., 2008). Upon pathogen infection or accumulation of SA, changes in cellular redox potential lead to the reduction of cysteines through the activity of thioredoxins (TRX-h3 and TRX-h5) and release of NPR1 monomers to localize to the nucleus (Tada et al., 2008).

Both NPR1 and TGA1 are well-described redox-regulated signaling compounds (Despres et al., 2003). TGA1 relies on the oxidation state of Cys residues to mediate the interaction with NPR1 (Despres et al., 2003). Interestingly, not only NPR1 but also TGA1 is S-nitrosylated after treatment with GSNO (Lindermayr, Sell, Muller, Leister, & Durner, 2010). Mass spectrometry analyses revealed that the Cys residues 260 and 266 of TGA1 are S-nitrosylated and S-glutathionylated (Lindermayr et al., 2010). GSNO protects TGA1 from oxygen-mediated modifications and enhances the DNA binding activity of TGA1 to the *as-1* element at *PR1* promoter in the presence of NPR1 (Lindermayr et al., 2010).

5.2 | Phosphorylation of NPR1

Besides modifications of the cysteine residues that affect the NPR1 oligomer–monomer switch, phosphorylation of NPR1 was also found to be required for its nuclear import and establishment of SAR. In the absence of SA accumulation, NPR1 is phosphorylated at Ser^{55}/Ser^{59} (Saleh et al., 2015). Inducers of SAR promote NPR1 dephosphorylation at Ser^{55}/Ser^{59} and promote phosphorylation at residues Ser^{11}/Ser^{15} (Spoel et al., 2009). However, the kinases responsible for phosphorylation at residues Ser^{11}/Ser^{15} or Ser^{55}/Ser^{59} have not been identified yet.

Due to the importance of NPR1, several kinases that phosphorylate NPR1 have been found. For instance, SnRK2.8 interacts with and phosphorylates NPR1, although it does not affect the NPR1 monomerization reaction (Lee et al., 2015). Phosphorylation within the C terminal NLS (Ser^{589}) by SnRK2.8 was found to be required for nuclear import and the establishment of SAR (Figure 4) (Lee et al., 2015). Furthermore, genetic evidence indicates that an additional threonine (Thr^{373}), which is identified by phosphoproteomic analysis of in vitro phosphorylated NPR1, might also be modified by SnRK2.8 as the npr1 (T373A) mutant fails to enter the nucleus (Lee et al., 2015). Another kinase that was found to interact with and phosphorylate NPR1 was PROTEIN KINASE SOS2-LIKE5 (PKS5), a

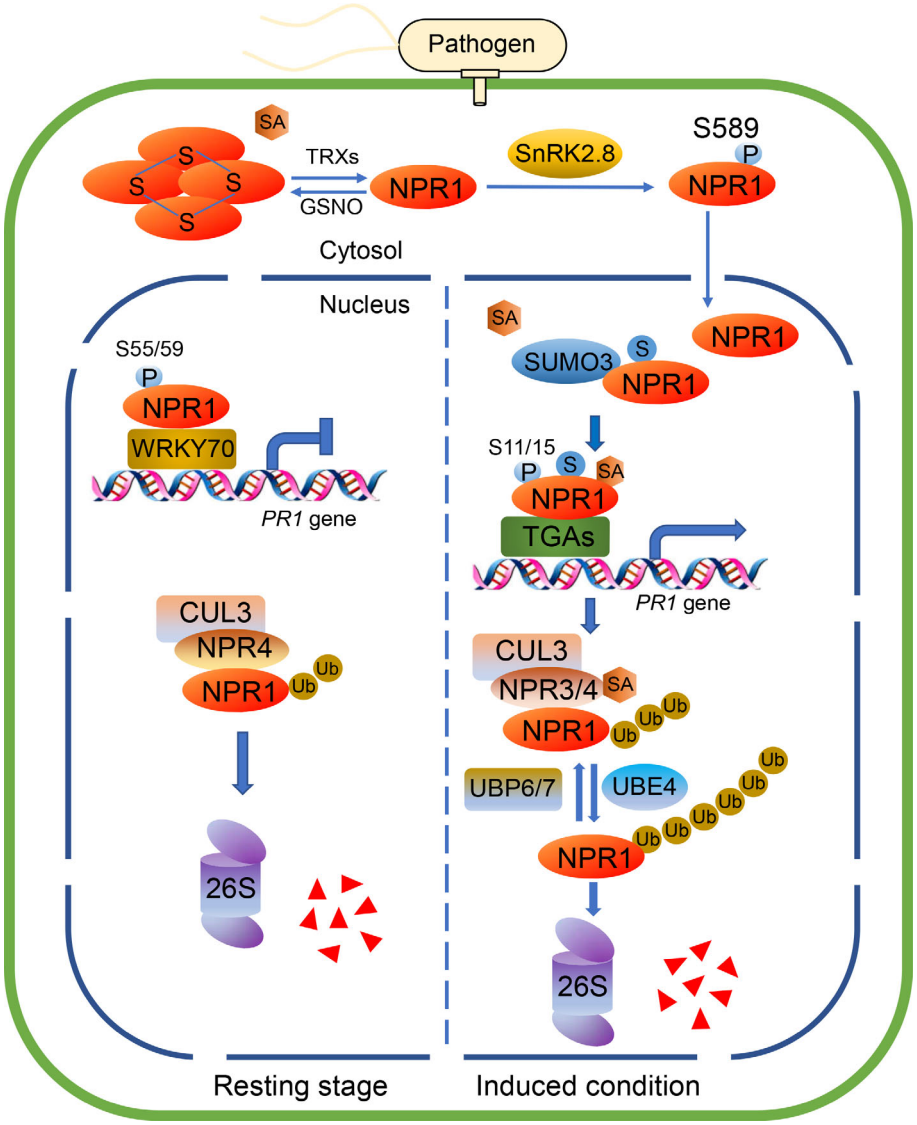

FIGURE 4 Post-translational regulation of NPR1 protein. At the resting stage, NPR1 mainly exists as oligomer in the cytosol. NPR1 is phosphorylated at Ser^{55}/Ser^{59} (S55/59) and interacts with WRKY70 to suppress *PR1* gene induction. NPR1 is constantly degraded by CUL3 and its adapter protein NPR4. Thioredoxins (TRXs) and GSNO mediate the transition of NPR1 between oligomeric and monomer state. Upon pathogen infection (induced condition), SA accumulates in the plant cell. SnRK2.8 phosphorylates NPR1 at S589 and facilitates its translocation from the cytosol to the nucleus. In the nucleus, SA accumulation promotes dephosphorylation of S55/59 through an unknown mechanism and induces the SUMOylation of NPR1 by SUMO3. SUMOylation promotes phosphorylation of NPR1 at Ser^{11}/Ser^{15} and the interaction between NPR1 and TGAs to facilitate *PR1* gene expression. The turnover of NPR1 protein is mediated by 26S proteasome (26S) complex through sequential polyubiquitination processes by CUL3 and E4 ligase UBE4. On the other hand, NPR1 deubiquitination process is mediated by ubiquitin-specific proteases UBP6 and UBP7, which are closely linked to 26S proteasome. P, phosphorylation; S, SUMOylation [Colour figure can be viewed at wileyonlinelibrary.com]

pathogen-responsive member of the sucrose non-fermenting 1 (SNF1)-related kinase 3 (SnRK3) subgroup (Xie, Zhou, Deng, & Guo, 2010). PKS5 phosphorylates the C-terminal region of NPR1. In *pks5* mutants, the expression level of two NPR1 target genes, *WRKY38* and *WRKY62*, is reduced and/or delayed (Xie et al., 2010). Despite this discovery, the phosphorylation site of NPR1 is still unknown. More recently, it was shown that MAP kinase MPK1 directly interacts with and phosphorylates NPR1 (J. Zhang et al., 2020). Meanwhile, MPK1 also mediates NPR1 monomerization (J. Zhang et al., 2020). Further research is required to understand the mechanism by which PKS5 and MPK1 phosphorylate NPR1 fully.

5.3 | Ubiquitination and de-ubiquitination of NPR1

NPR1 activity is tightly regulated by post-translational degradation. Proteasome-mediated turnover of NPR1 within the nucleus is a requirement for the full induction of target genes and the establishment of SAR (Spoel et al., 2009). In the absence of pathogen challenge, NPR1 is continuously cleared from the nucleus by the proteasome (Spoel et al., 2009). Inducers of SAR promote NPR1 phosphorylation at residues Ser^{11}/Ser^{15} and facilitate its recruitment to a CUL3-based ubiquitin ligase (Spoel et al., 2009). Interestingly, abscisic acid (ABA) also influences cellular NPR1 protein levels. ABA promotes NPR1 degradation via the 26S proteasome pathway, whereas SA may protect NPR1 from ABA-promoted degradation through phosphorylation (Ding, Dommel, & Mou, 2016). NPR1 does not interact directly with CUL3, although NPR1 could be pulled down with an antibody against CUL3A or co-immunoprecipitates with CUL3 in *N. benthamiana* extracts (Dieterle et al., 2005; Spoel et al., 2009; Zavaliev et al., 2020). Interestingly, a bacteria effector AvrPtoB, an E3 ligase, also targets NPR1 for degradation via the host 26S proteasome pathway, thereby subverting plant immunity (H. Chen et al., 2017). In rice, OsCUL3a interacts with and degrades OsNPR1, which acts as a positive regulator of cell death in rice (Q. Liu et al., 2017). The function of NPR1 in plant immunity has been revealed in other species as

well (X. K. Chen et al., 2012; Malnoy, Jin, Borejsza-Wysocka, He, & Aldwinckle, 2007; L. Wang et al., 2017; Zhang et al., 2012; Y.-m. Zhang, Ni, Ma, & Qiu, 2013). An investigation into whether or not NPR1 proteins in other species are also degraded by E3 ligase activity to regulate plant immunity would prove to be interesting.

The turnover of NPR1 protein is mediated by the 26S proteasome complex through sequential polyubiquitination processes by E3 ligase CUL3 and E4 ligase UBE4 (Figure 4) (Skelly, Furniss, Grey, Wong, & Spoel, 2019). Initial ubiquitin modifications mediated by CUL3 enhance target gene expression to maximum levels (Skelly et al., 2019). UBE4 is involved in the polyubiquitination of NPR1. Long-chain polyubiquitination of NPR1 mediated by UBE4 promotes its proteasome-mediated degradation and inactivates target gene expression (Skelly et al., 2019). The complexity of the ubiquitin-dependent post-translational regulation of NPR1 was further revealed by the identification of ubiquitin-specific protease UBP6 and UBP7 that deubiquitinated NPR1 (Figure 4). Knockout of *UBP6* and *UBP7* resulted in an enhanced turnover and decreased transcriptional output of NPR1 (Skelly et al., 2019). Therefore, ubiquitin chain extension and trimming activities can fine-tune transcriptional outputs of transcriptional coactivator NPR1.

5.4 | SUMOylation of NPR1

In addition, the activity of NPR1 is also regulated by SUMOylation. Small ubiquitin-like modifier 3 (SUMO3) interacts with and SUMOylates NPR1 following SA treatment (Saleh et al., 2015). SUMO-interaction motif (VIL)-(VIL)-x-(VIL) found within the ankyrin repeat domain of NPR1 is required for the interaction between NPR1 and SUMO3 (Saleh et al., 2015). In the absence of SA accumulation, NPR1 is phosphorylated at Ser^{55}/Ser^{59}, which blocks SUMOylation and promotes interaction with WRKY70 To repress *PR1* expression (Saleh et al., 2015). Upon induction, Ser^{55}/Ser^{59} of NPR1 is likely dephosphorylated, allowing NPR1 to become SUMOylated. SUMOylation of NPR1 activates defense gene expression by switching NPR1's association with the WRKY transcription repressors to TGA transcription activators. In addition, modification of NPR1 by SUMO3 is required for phosphorylation at Ser^{11}/Ser^{15} to form a signal amplification loop to generate more activated NPR1 (Figure 4) (Saleh et al., 2015). Thus, the interplay between SUMOylation and phosphorylation fine-tunes NPR1 activity and determines the fate of NPR1.

6 | CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Transcriptional regulation of NPR1 plays a vital role in SA signaling. Since only a few transcription factors that regulate NPR1 promoter activity have been found, some transcription factors are still to be identified. *NPR1* promoter contains a *TL1* (CTGAAGAAGAA) element; therefore, the *NPR1* gene expression may be regulated by *TL1*-binding transcription factor TBF1 (Pajerowska-Mukhtar et al., 2012). Research has shown that CDK8 functions as a bridge between WRKY transcription factors that bind to NPR1 promoter and RNA polymerase II (J. Chen et al., 2019). CDK8 kinase module subunits MED12 and MED13 also positively regulate NPR1 gene expression (J. Chen et al., 2019). An investigation into whether or not MED12 and MED13 also associate with NPR1 promoters through transcription factors, such as WRKY18, WRKY6 or others that are yet to be identified would be interesting (Figure 3). In addition, SARD1 and CBP60g have been found to bind to the promoter of *NPR1* gene through ChIP assay (T. Sun et al., 2015); however, the underlying molecular mechanism remains cryptic (Figure 3).

Post-transcriptional modifications of NPR1 have been extensively studied. Despite the extensive research, there are still some basic questions that remain to be answered. NPR1 interacts with transcription factors, such as TGAs, to regulate defense gene expression; however, how exactly is the transcription coactivity of NPR1 orchestrated? What genes do NPR1 control during specific time points of immune response? How do specific post-transcriptional modifications of NPR1 affect its interaction with TGAs and TEOSINTE BRANCHED 1, CYCLOIDEA, PCF1 (TCP) transcription factors that have been shown to regulate *PR5* gene expression (Li et al., 2018)? A previous study showed that NPR1 forms a protein complex with HAC1 and TGAs to regulate *PR1* gene expression. Thus, NPR1 may interact with other chromatin remodeling proteins as well, which warrants further investigation. In addition, ChIP assay using NPR1 transgenic plant will help us further understand the regulatory role of NPR1 in the expression of defense genes.

Phosphorylation has been shown to regulate multiple NPR1 functions. Two members of the SnRK family of kinases have been shown to interact with and phosphorylate NPR1 (Lee et al., 2015; Xie et al., 2010). However, the kinase(s) responsible for Ser^{11}/Ser^{15} and Ser^{55}/Ser^{59} phosphorylation has not yet been identified. NPR1 contains multiple sites that are potentially phosphorylated (Withers & Dong, 2016). Therefore, further identification of those sites to understand the phosphorylation events within the NPR1 protein would be interesting. Recent studies have suggested that NPR1 is also dephosphorylated (Saleh et al., 2015). However, no phosphatases that directly interact with and regulate NPR1 have been discovered.

The post-translational regulation of NPR1 is well studied. For instance, NPR1 could be degraded by the 26S proteasome pathway by several E3 ligases. However, in other scenarios, the components which could protect NPR1 from degradation still need to be identified. Furthermore, there is no current knowledge if all NPR1 paralogues would undergo similar biochemical processes to NPR1 in response to SA accumulation, such as the transition from oligomer to monomer, translocation from cytosol to nuclear, polyubiquitination and rapid protein turnover. As for the biochemical nature of the NPR proteins, although the crystal structure of NPR4 SBC has been revealed, the crystal structures of full-length NPR1/3/4 are still mysteries. The structural determination of full-length NPR1/3/4 in its modified states and/or bound to SA would provide the ultimate understanding of the physical dynamics of NPR1/3/4.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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REFERENCES

Attaran, E., Zeier, T. E., Griebel, T., & Zeier, J. (2009). Methyl salicylate production and jasmonate signaling are not essential for systemic acquired resistance in Arabidopsis. *The Plant Cell*, *21*(3), 954–971.

Barlev, N. A., Liu, L., Chehab, N. H., Mansfield, K., Harris, K. G., Halazonetis, T. D., & Berger, S. L. (2001). Acetylation of p53 activates transcription through recruitment of coactivators/histone acetyltransferases. *Molecular Cell*, *8*(6), 1243–1254.

Boyle, P., Le Su, E., Rochon, A., Shearer, H. L., Murmu, J., Chu, J. Y., … Despres, C. (2009). The BTB/POZ domain of the Arabidopsis disease resistance protein NPR1 interacts with the repression domain of TGA2 to negate its function. *The Plant Cell*, *21*(11), 3700–3713.

Cao, H., Bowling, S. A., Gordon, A. S., & Dong, X. (1994). Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance. *The Plant Cell*, *6*(11), 1583–1592.

Chai, J., Liu, J., Zhou, J., & Xing, D. (2014). Mitogen-activated protein kinase 6 regulates NPR1 gene expression and activation during leaf senescence induced by salicylic acid. *Journal of Experimental Botany*, *65*(22), 6513–6528.

Chen, H., Chen, J., Li, M., Chang, M., Xu, K., Shang, Z., … Fu, Z. Q. (2017). A bacterial type III effector targets the master regulator of salicylic acid signaling, NPR1, to subvert plant immunity. *Cell Host & Microbe*, *22*(6), 777–788.

Chen, J., Clinton, M., Qi, G., Wang, D., Liu, F., & Fu, Z. Q. (2020). Reprogramming and remodeling: Transcriptional and epigenetic regulation of salicylic acid-mediated plant defense. *Journal of Experimental Botany*, *71*(17), 5256–5268.

Chen, J., Mohan, R., Zhang, Y., Li, M., Chen, H., Palmer, I. A., … Fu, Z. Q. (2019). NPR1 promotes its own and target gene expression in plant defense by recruiting CDK8. *Plant Physiology*, *181*(1), 289–304.

Chen, X. K., Zhang, J. Y., Zhang, Z., Du, X. L., Du, B. B., & Qu, S. C. (2012). Overexpressing MhNPR1 in transgenic Fuji apples enhances resistance to apple powdery mildew. *Molecular Biology Reports*, *39*(8), 8083–8089.

Chen, Y. C., Holmes, E. C., Rajniak, J., Kim, J. G., Tang, S., Fischer, C. R., … Sattely, E. S. (2018). N-hydroxy-pipecolic acid is a mobile metabolite that induces systemic disease resistance in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(21), E4920–E4929.

Chen, Z., Ricigliano, J. W., & Klessig, D. F. (1993). Purification and characterization of a soluble salicylic acid-binding protein from tobacco. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(20), 9533–9537.

Chinchilla, D., Bauer, Z., Regenass, M., Boller, T., & Felix, G. (2006). The Arabidopsis receptor kinase FLS2 binds flg22 and determines the specificity of flagellin perception. *The Plant Cell*, *18*(2), 465–476.

Chinchilla, D., Zipfel, C., Robatzek, S., Kemmerling, B., Nurnberger, T., Jones, J. D., … Boller, T. (2007). A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature*, *448*(7152), 497–500.

Collier, S. M., & Moffett, P. (2009). NB-LRRs work a "bait and switch" on pathogens. *Trends in Plant Science*, *14*(10), 521–529.

Delaney, T. P., Friedrich, L., & Ryals, J. A. (1995). Arabidopsis signal transduction mutant defective in chemically and biologically induced disease resistance. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(14), 6602–6606.

Delaney, T. P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., … Ryals, J. (1994). A central role of salicylic Acid in plant disease resistance. *Science*, *266*(5188), 1247–1250.

Despres, C., Chubak, C., Rochon, A., Clark, R., Bethune, T., Desveaux, D., & Fobert, P. R. (2003). 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. *The Plant Cell*, *15*(9), 2181–2191.

Despres, C., DeLong, C., Glaze, S., Liu, E., & Fobert, P. R. (2000). The Arabidopsis NPR1/NIM1 protein enhances the DNA binding activity of a subgroup of the TGA family of bZIP transcription factors. *The Plant Cell*, *12*(2), 279–290.

Desveaux, D., Singer, A. U., Wu, A. J., McNulty, B. C., Musselwhite, L., Nimchuk, Z., … Dangl, J. L. (2007). Type III effector activation via nucleotide binding, phosphorylation, and host target interaction. *PLoS Pathogens*, *3*(3), e48.

Devadas, S. K., & Raina, R. (2002). Preexisting systemic acquired resistance suppresses hypersensitive response-associated cell death in Arabidopsis hrl1 mutant. *Plant Physiology*, *128*(4), 1234–1244.

Dieterle, M., Thomann, A., Renou, J. P., Parmentier, Y., Cognat, V., Lemonnier, G., … Genschik, P. (2005). Molecular and functional characterization of Arabidopsis Cullin 3A. *The Plant Journal*, *41*(3), 386–399.

Ding, Y., Dommel, M., & Mou, Z. (2016). Abscisic acid promotes proteasome-mediated degradation of the transcription coactivator NPR1 in *Arabidopsis thaliana*. *The Plant Journal*, *86*(1), 20–34.

Ding, Y., Sun, T., Ao, K., Peng, Y., Zhang, Y., Li, X., & Zhang, Y. (2018). Opposite roles of salicylic acid receptors NPR1 and NPR3/NPR4 in transcriptional regulation of plant immunity. *Cell*, *173*(6), 1454–1467 e1415.

Dodds, P. N., & Rathjen, J. P. (2010). Plant immunity: Towards an integrated view of plant-pathogen interactions. *Nature Reviews Genetics*, *11*(8), 539–548.

Du, H., & Klessig, D. F. (1997). Identification of a soluble, high-affinity salicylic acid-binding protein in tobacco. *Plant Physiology*, *113*(4), 1319–1327.

Falk, A., Feys, B. J., Frost, L. N., Jones, J. D., Daniels, M. J., & Parker, J. E. (1999). EDS1, an essential component of R gene-mediated disease resistance in Arabidopsis has homology to eukaryotic lipases. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(6), 3292–3297.

Feys, B. J., Moisan, L. J., Newman, M. A., & Parker, J. E. (2001). Direct interaction between the Arabidopsis disease resistance signaling proteins, EDS1 and PAD4. *EMBO Journal*, *20*(19), 5400–5411.

Fu, Z. Q., & Dong, X. (2013). Systemic acquired resistance: Turning local infection into global defense. *Annual Review of Plant Biology*, *64*, 839–863.

Fu, Z. Q., Yan, S., Saleh, A., Wang, W., Ruble, J., Oka, N., … Dong, X. (2012). NPR3 and NPR4 are receptors for the immune signal salicylic acid in plants. *Nature*, *486*(7402), 228–232.

Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., … Ryals, J. (1993). Requirement of salicylic Acid for the induction of systemic acquired resistance. *Science*, *261*(5122), 754–756.

Gomez-Gomez, L., & Boller, T. (2002). Flagellin perception: A paradigm for innate immunity. *Trends in Plant Science*, *7*(6), 251–256.

Hartmann, M., Zeier, T., Bernsdorff, F., Reichel-Deland, V., Kim, D., Hohmann, M., … Zeier, J. (2018). Flavin monooxygenase-generated N-hydroxypipecolic acid is a critical element of plant systemic immunity. *Cell*, *173*(2), 456–469.

Jin, H., Choi, S. M., Kang, M. J., Yun, S. H., Kwon, D. J., Noh, Y. S., & Noh, B. (2018). Salicylic acid-induced transcriptional reprogramming by the HAC-NPR1-TGA histone acetyltransferase complex in Arabidopsis. *Nucleic Acids Research*, *46*(22), 11712–11725.

Jones, J. D., & Dangl, J. L. (2006). The plant immune system. *Nature*, *444*(7117), 323–329.

Kim, H. S., & Delaney, T. P. (2002). Over-expression of TGA5, which encodes a bZIP transcription factor that interacts with NIM1/NPR1, confers SAR-independent resistance in *Arabidopsis thaliana* to Peronospora parasitica. *The Plant Journal*, *32*(2), 151–163.

Kim, Y., Gilmour, S. J., Chao, L., Park, S., & Thomashow, M. F. (2020). Arabidopsis CAMTA transcription factors regulate pipecolic acid biosynthesis and priming of immunity genes. *Molecular Plant*, *13*(1), 157–168.

Kinkema, M., Fan, W., & Dong, X. (2000). Nuclear localization of NPR1 is required for activation of PR gene expression. *The Plant Cell*, *12*(12), 2339–2350.

Kumar, D., & Klessig, D. F. (2003). High-affinity salicylic acid-binding protein 2 is required for plant innate immunity and has salicylic acid-stimulated lipase activity. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(26), 16101–16106.

Kunkel, B. N., Bent, A. F., Dahlbeck, D., Innes, R. W., & Staskawicz, B. J. (1993). RPS2, an Arabidopsis disease resistance locus specifying recognition of Pseudomonas syringae strains expressing the avirulence gene avrRpt2. *The Plant Cell*, *5*(8), 865–875.

Lee, H. J., Park, Y. J., Seo, P. J., Kim, J. H., Sim, H. J., Kim, S. G., & Park, C. M. (2015). Systemic immunity requires SnRK2.8-mediated nuclear import of NPR1 in Arabidopsis. *The Plant Cell*, *27*(12), 3425–3438.

Li, M., Chen, H., Chen, J., Chang, M., Palmer, I. A., Gassmann, W., … Fu, Z. Q. (2018). TCP transcription factors interact with NPR1 and contribute redundantly to systemic acquired resistance. *Frontiers in Plant Science*, *9*, 1153.

Lim, G. H., Liu, H., Yu, K., Liu, R., Shine, M. B., Fernandez, J., … Kachroo, P. (2020). The plant cuticle regulates apoplastic transport of salicylic acid during systemic acquired resistance. *Science Advances*, *6*(19), eaaz0478.

Lim, G. H., Shine, M. B., de Lorenzo, L., Yu, K., Cui, W., Navarre, D., … Kachroo, P. (2016). Plasmodesmata localizing proteins regulate transport and signaling during systemic acquired immunity in plants. *Cell Host & Microbe*, *19*(4), 541–549.

Lindermayr, C., Sell, S., Muller, B., Leister, D., & Durner, J. (2010). Redox regulation of the NPR1-TGA1 system of *Arabidopsis thaliana* by nitric oxide. *The Plant Cell*, *22*(8), 2894–2907.

Liu, L., Sonbol, F. M., Huot, B., Gu, Y., Withers, J., Mwimba, M., … Dong, X. (2016). Salicylic acid receptors activate jasmonic acid signalling through a non-canonical pathway to promote effector-triggered immunity. *Nature Communications*, *7*, 13099.

Liu, Q., Ning, Y., Zhang, Y., Yu, N., Zhao, C., Zhan, X., … Cao, L. (2017). OsCUL3a negatively regulates cell death and immunity by degrading OsNPR1 in rice. *The Plant Cell*, *29*(2), 345–359.

Mackey, D., Holt, B. F., 3rd, Wiig, A., & Dangl, J. L. (2002). RIN4 interacts with Pseudomonas syringae type III effector molecules and is required for RPM1-mediated resistance in Arabidopsis. *Cell*, *108*(6), 743–754.

Malamy, J., Carr, J. P., Klessig, D. F., & Raskin, I. (1990). Salicylic Acid: A likely endogenous signal in the resistance response of tobacco to viral infection. *Science*, *250*(4983), 1002–1004.

Malnoy, M., Jin, Q., Borejsza-Wysocka, E. E., He, S. Y., & Aldwinckle, H. S. (2007). Overexpression of the apple MpNPR1 gene confers increased disease resistance in Malus x domestica. *Molecular Plant-Microbe Interactions*, *20*(12), 1568–1580.

Manohar, M., Tian, M., Moreau, M., Park, S. W., Choi, H. W., Fei, Z., … Klessig, D. F. (2015). Identification of multiple salicylic acid-binding proteins using two high throughput screens. *Frontiers in Plant Science*, *5*, 777.

Métraux, J. P., Signer, H., Ryals, J., Ward, E., Wyss-Benz, M., Gaudin, J., … Inverardi, B. (1990). Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. *Science*, *250*(4983), 1004–1006.

Mou, Z., Fan, W., & Dong, X. (2003). Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. *Cell*, *113*(7), 935–944.

Návarová, H., Bernsdorff, F., Döring, A.-C., & Zeier, J. (2012). Pipecolic acid, an endogenous mediator of defense amplification and priming, is a critical regulator of inducible plant immunity. *The Plant Cell*, *24*(12), 5123–5141.

Nawrath, C., & Metraux, J. P. (1999). Salicylic acid induction-deficient mutants of Arabidopsis express PR-2 and PR-5 and accumulate high levels of camalexin after pathogen inoculation. *The Plant Cell*, *11*(8), 1393–1404.

Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B. H., & Nakatani, Y. (1996). The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell*, *87*(5), 953–959.

Pajerowska-Mukhtar, K. M., Wang, W., Tada, Y., Oka, N., Tucker, C. L., Fonseca, J. P., & Dong, X. N. (2012). The HSF-like transcription factor TBF1 is a major molecular switch for plant growth-to-defense transition. *Current Biology*, *22*(2), 103–112.

Park, S. W., Kaimoyo, E., Kumar, D., Mosher, S., & Klessig, D. F. (2007). Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. *Science*, *318*(5847), 113–116.

Pieterse, C. M., Leon-Reyes, A., Van der Ent, S., & Van Wees, S. C. (2009). Networking by small-molecule hormones in plant immunity. *Nature Chemical Biology*, *5*(5), 308–316.

Qi, G., Chen, J., Chang, M., Chen, H., Hall, K., Korin, J., … Fu, Z. Q. (2018). Pandemonium breaks out: Disruption of salicylic acid-mediated defense by plant pathogens. *Molecular Plant.*, *11*(12), 1427–1439.

Radojicic, A., Li, X., & Zhang, Y. (2018). Salicylic acid: A double-edged sword for programed cell death in plants. *Frontiers in Plant Science*, *9*, 1133.

Rate, D. N., & Greenberg, J. T. (2001). The Arabidopsis aberrant growth and death2 mutant shows resistance to Pseudomonas syringae and reveals a role for NPR1 in suppressing hypersensitive cell death. *The Plant Journal*, *27*(3), 203–211.

Rochon, A., Boyle, P., Wignes, T., Fobert, P. R., & Despres, C. (2006). The coactivator function of Arabidopsis NPR1 requires the core of its BTB/POZ domain and the oxidation of C-terminal cysteines. *The Plant Cell*, *18*(12), 3670–3685.

Saleh, A., Withers, J., Mohan, R., Marques, J., Gu, Y., Yan, S., … Dong, X. (2015). Posttranslational modifications of the master transcriptional regulator NPR1 enable dynamic but tight control of plant immune responses. *Cell Host & Microbe*, *18*(2), 169–182.

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., & Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution*, *3*(3), 430–439.

Skelly, M. J., Furniss, J. J., Grey, H., Wong, K. W., & Spoel, S. H. (2019). Dynamic ubiquitination determines transcriptional activity of the plant immune coactivator NPR1. *Elife*, *8*, e47005.

Slaymaker, D. H., Navarre, D. A., Clark, D., del Pozo, O., Martin, G. B., & Klessig, D. F. (2002). The tobacco salicylic acid-binding protein 3 (SABP3) is the chloroplast carbonic anhydrase, which exhibits antioxidant activity and plays a role in the hypersensitive defense response. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(18), 11640–11645.

Spoel, S. H., Mou, Z., Tada, Y., Spivey, N. W., Genschik, P., & Dong, X. (2009). Proteasome-mediated turnover of the transcription coactivator NPR1 plays dual roles in regulating plant immunity. *Cell*, *137*(5), 860–872.

Sun, T., Huang, J., Xu, Y., Verma, V., Jing, B., Sun, Y., … Li, X. (2020). Redundant CAMTA transcription factors negatively regulate the biosynthesis of salicylic acid and N-hydroxypipecolic acid by modulating the expression of SARD1 and CBP60g. *Molecular Plant*, *13*(1), 144–156.

Sun, T., Zhang, Y., Li, Y., Zhang, Q., Ding, Y., & Zhang, Y. (2015). ChIP-seq reveals broad roles of SARD1 and CBP60g in regulating plant immunity. *Nature Communications*, *6*, 10159.

Sun, Y., Zhu, Y.-X., Balint-Kurti, P. J., & Wang, G.-F. (2020). Fine-tuning immunity: Players and regulators for plant NLRs. *Trends in Plant Science*, *25*(7), 695–713.

Tada, Y., Spoel, S. H., Pajerowska-Mukhtar, K., Mou, Z., Song, J., Wang, C., … Dong, X. (2008). Plant immunity requires conformational changes [corrected] of NPR1 via S-nitrosylation and thioredoxins. *Science*, *321* (5891), 952–956.

Tateda, C., Zhang, Z., Shrestha, J., Jelenska, J., Chinchilla, D., & Greenberg, J. T. (2014). Salicylic acid regulates Arabidopsis microbial pattern receptor kinase levels and signaling. *The Plant Cell*, *26*(10), 4171–4187.

Tsuda, K., Sato, M., Glazebrook, J., Cohen, J. D., & Katagiri, F. (2008). Interplay between MAMP-triggered and SA-mediated defense responses. *The Plant Journal*, *53*(5), 763–775.

Tsuda, K., Sato, M., Stoddard, T., Glazebrook, J., & Katagiri, F. (2009). Network properties of robust immunity in plants. *PLoS Genetics*, *5*(12), e1000772.

van Loon, L. C., Rep, M., & Pieterse, C. M. (2006). Significance of inducible defense-related proteins in infected plants. *Annual Review of Phytopathology*, *49*(44), 135–162.

van Wersch, S., Tian, L., Hoy, R., & Li, X. (2020). Plant NLRs: The whistleblowers of plant immunity. *Plant Communications*, *1*(1), 100016.

Wang, L., Guo, Z., Zhang, Y., Wang, Y., Yang, G., Yang, L., … Xie, Z. (2017). Overexpression of LhSorNPR1, a NPR1-like gene from the oriental hybrid lily 'Sorbonne', conferred enhanced resistance to Pseudomonas syringae pv. tomato DC3000 in Arabidopsis. *Physiology and Molecular Biology of Plants*, *23*(4), 793–808.

Wang, W., Withers, J., Li, H., Zwack, P. J., Rusnac, D.-V., Shi, H., … Zheng, N. (2020). Structural basis of salicylic acid perception by Arabidopsis NPR proteins. *Nature*, *586*, 311–316. https://doi.org/10.1038/s41586-020-2596-y

White, R. F. (1979). Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. *Virology*, *99*(2), 410–412.

Withers, J., & Dong, X. (2016). Posttranslational modifications of NPR1: A single protein playing multiple roles in plant immunity and physiology. *PLoS Pathogens*, *12*(8), e1005707.

Wu, Y., Zhang, D., Chu, J. Y., Boyle, P., Wang, Y., Brindle, I. D., … Despres, C. (2012). The Arabidopsis NPR1 protein is a receptor for the plant defense hormone salicylic acid. *Cell Reports*, *1*(6), 639–647.

Xie, C., Zhou, X., Deng, X., & Guo, Y. (2010). PKS5, a SNF1-related kinase, interacts with and phosphorylates NPR1, and modulates expression of WRKY38 and WRKY62. *Journal of Genetics and Genomics*, *37*(6), 359–369.

Yu, D., Chen, C., & Chen, Z. (2001). Evidence for an important role of WRKY DNA binding proteins in the regulation of NPR1 gene expression. *The Plant Cell*, *13*(7), 1527–1540.

Yuan, H. M., Liu, W. C., & Lu, Y. T. (2017). CATALASE2 coordinates SA-mediated repression of both auxin accumulation and JA biosynthesis in plant defenses. *Cell Host & Microbe*, *21*(2), 143–155.

Zavaliev, R., Mohan, R., Chen, T., & Dong, X. (2020). Formation of NPR1 condensates promotes cell survival during the plant immune response. *Cell*, *182*, 1–16.

Zhang, J., Gao, J., Zhu, Z., Song, Y., Wang, X., Wang, X., & Zhou, X. (2020). MKK4/MKK5-MPK1/MPK2 cascade mediates SA-activated leaf senescence via phosphorylation of NPR1 in Arabidopsis. *Plant Molecular Biology*, *102*(4-5), 463–475.

Zhang, J. Y., Qiao, Y. S., Lv, D., Gao, Z. H., Qu, S. C., & Zhang, Z. (2012). *Malus hupehensis* NPR1 induces pathogenesis-related protein gene expression in transgenic tobacco. *Plant Biology (Stuttgart, Germany)*, *14* (Suppl 1), 46–56.

Zhang, X., Wang, C., Zhang, Y., Sun, Y., & Mou, Z. (2012). The Arabidopsis mediator complex subunit16 positively regulates salicylate-mediated systemic acquired resistance and jasmonate/ethylene-induced defense pathways. *The Plant Cell*, *24*(10), 4294–4309.

Zhang, Y., Cheng, Y. T., Qu, N., Zhao, Q., Bi, D., & Li, X. (2006). Negative regulation of defense responses in Arabidopsis by two NPR1 paralogs. *The Plant Journal*, *48*(5), 647–656.

Zhang, Y., & Li, X. (2019). Salicylic acid: Biosynthesis, perception, and contributions to plant immunity. *Current Opinion in Plant Biology*, *50*, 29–36.

Zhang, Y.-m., Ni, X.-l., Ma, H.-q., & Qiu, W. (2013). Characterization of NPR1 genes from norton and cabernet sauvignon grapevine. *Journal of Integrative Agriculture*, *12*(7), 1152–1161.

Zhou, J. M., Trifa, Y., Silva, H., Pontier, D., Lam, E., Shah, J., & Klessig, D. F. (2000). NPR1 differentially interacts with members of the TGA/OBF family of transcription factors that bind an element of the PR-1 gene required for induction by salicylic acid. *Molecular Plant-Microbe Interactions*, *13*(2), 191–202.

Zipfel, C., Robatzek, S., Navarro, L., Oakeley, E. J., Jones, J. D., Felix, G., & Boller, T. (2004). Bacterial disease resistance in Arabidopsis through flagellin perception. *Nature*, *428*(6984), 764–767.

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