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Review

NPR1, a key immune regulator for plant survival under biotic and abiotic stresses

Raul Zavaliev[1,*] and Xinnian Dong[1,*]
[1]Howard Hughes Medical Institute, Department of Biology, Duke University, Durham, NC 27708, USA
*Correspondence: raul.zavaliev@duke.edu (R.Z.), xdong@duke.edu (X.D.)
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SUMMARY

Nonexpressor of pathogenesis-related genes 1 (NPR1) was discovered in *Arabidopsis* as an activator of salicylic acid (SA)-mediated immune responses nearly 30 years ago. How NPR1 confers resistance against a variety of pathogens and stresses has been extensively studied; however, only in recent years have the underlying molecular mechanisms been uncovered, particularly NPR1's role in SA-mediated transcriptional reprogramming, stress protein homeostasis, and cell survival. Structural analyses ultimately defined NPR1 and its paralogs as SA receptors. The SA-bound NPR1 dimer induces transcription by bridging two TGA transcription factor dimers, forming an enhanceosome. Moreover, NPR1 orchestrates its multiple functions through the formation of distinct nuclear and cytoplasmic biomolecular condensates. Furthermore, NPR1 plays a central role in plant health by regulating the crosstalk between SA and other defense and growth hormones. In this review, we focus on these recent advances and discuss how NPR1 can be utilized to engineer resistance against biotic and abiotic stresses.

INTRODUCTION

Plants deploy different defense mechanisms to fight pathogen infection and survive extreme environmental stress. Upon pathogen challenge, complex multilayered responses occur at the infection site, in neighboring uninfected cells, and eventually at the whole plant level. The first line of active defense in plants is through the recognition of microbe/damage-associated molecular patterns (MAMPs/DAMPs) by cell-surface pattern-recognition receptors (PRRs).[1,2] Adapted pathogens can overcome this pattern-triggered immunity (PTI) by delivering effectors into plant tissues to promote virulence. The second line of defense is triggered when the activity of these effectors is detected by the host intracellular nucleotide-binding leucine-rich repeat (NB-LRR) immune receptors.[3–6] This effector-triggered immunity (ETI) is a high-amplitude defense response that often culminates in programmed cell death (PCD) at the site of infection and production of the plant immune hormone salicylic acid (SA). The increase in SA not only promotes basal resistance at the infection site[7] and the survival of adjacent cells[8] but is also needed for priming distal tissues for the long-lasting, broad-spectrum immunity termed systemic acquired resistance (SAR).[9] These responses to SA are characterized by transcriptional reprogramming that leads to the coordinated induction of a great number of defense genes encoding antimicrobial peptides and cellular machineries required for immune proteome homeostasis.[8,10]

SA signaling requires nonexpressor of pathogenesis-related (PR) genes 1 (NPR1), which was first identified in the model plant *Arabidopsis thaliana*.[11–14] Consistent with its central role in plant defense, overexpression of *Arabidopsis* NPR1 in diverse plant species, including many crops, enhances resistance against a variety of pathogens.[15–33] This raises the following questions: (1) what is the intrinsic property of NPR1 that allows it to protect plants against such a wide variety of stresses? And (2) how can we use NPR1 to engineer broad-spectrum disease resistance in crops as an alternative to the traditional pathogen-specific resistance? Recent advances in addressing these questions will be highlighted in this review.

SAR AND ITS SIGNALING MOLECULE SA

SAR was observed almost a century ago in the search for ways to "vaccinate" plants against phytopathogens.[34] However, the first comprehensive experimental demonstration of SAR was reported in 1961 by Frank Ross[35] using *Tobacco Mosaic Virus* (TMV), which, upon recognition by the host plant, triggers a localized PCD at the site of infection. This local response (later known as ETI) enhances resistance to secondary infection by the same or a different virus in systemic tissues defined as the uninoculated half of the leaf or the distal leaves. However, in contrast to vaccination in vertebrates, SAR is not pathogen specific but rather a broad-spectrum immune response. The advent of molecular genetic tools in plant research revived the interest in SAR due to its potential use in managing crop diseases in agriculture. As a result, numerous examples of SAR have been reported and the chemical, biochemical, and genetic basis of this immune mechanism investigated.[36,37] It was observed that upon SAR induction, tissues distant to the initial infection site accumulate several defense signals, which are essential for the establishment of SAR.[9,38,39] The earliest signal molecule identified was SA, whose biosynthesis is induced in local infected and systemic naive tissues.[40–42] In *Arabidopsis*, this is primarily through activation of the *isochorismate synthase 1* gene (*ICS1*).[43] Interestingly, grafting experiments showed that the

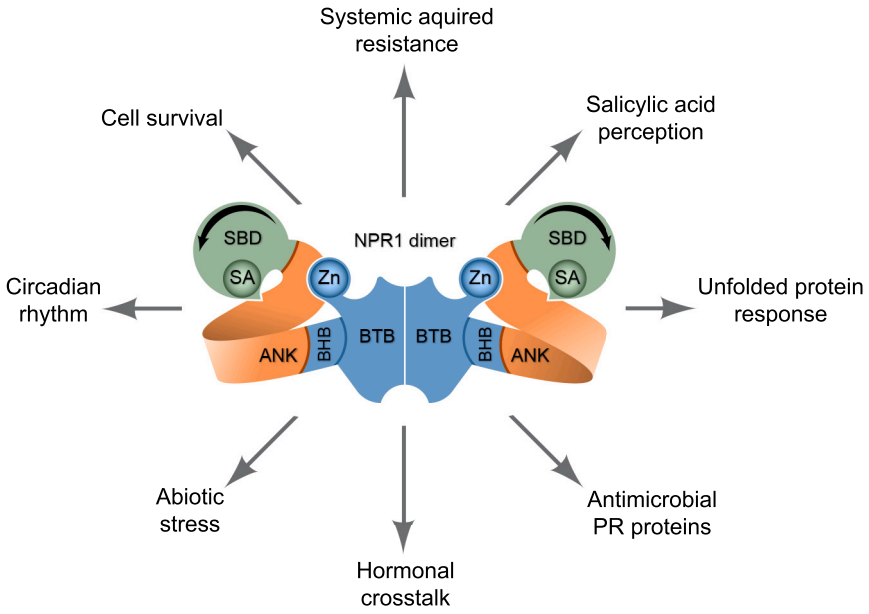

Figure 1. Processes regulated by NPR1
A spatial model of the NPR1 dimer structure is shown with domains and cofactors indicated. BTB, broad-complex, tramtrack, and Bric-à-brac; BHB, BACK (BTB and carboxyterminal Kelch) helix bundle; ANK, ankyrin repeat; SBD, SA-binding domain; Zn, zinc finger.

locally produced SA is not a mobile signal. Instead, *de novo* synthesis of SA in systemic tissues is required for SAR.[44] However, a direct connection between local induction and systemic SA synthesis was only recently established.[45,46] It was found that H_2O_2, produced by the cell-surface respiratory burst oxidase homolog D (RBOHD), is a mobile signal that mediates the sulfenylation of the CCA1 HIKING EXPEDITION (CHE) transcription factor (TF) to induce its binding to the *ICS1* gene promoter and trigger SA synthesis. Mutating the conserved H_2O_2-sensitive cysteine residue in CHE specifically compromises systemic SA synthesis and abolishes SAR.[46] Other mobile signaling molecules have also been reported to function synergistically with SA to confer fully fledged systemic resistance.[47–51] Among them, pipecolic acid (Pip) was found to accumulate in both local and systemic tissues and vasculature upon pathogen challenge, although in systemic tissues, it is dispensable for SAR.[52] It is possible that Pip and/or its derivative N-hydroxypipecolic acid (NHP)[47,53] serves as an amplifier of the SA signal.[46,54–56] Consistent with this hypothesis, both signals were shown to be inactivated by the same glycosyltransferase.[57–59]

The wide use of various chemical inducers of plant immunity, including analogs of SA, to boost broad-spectrum resistance in crops[60] further supports the essential role of SA and associated systemic metabolites in the activation of SAR. Moreover, NHP signaling was found to require NPR1,[61,62] suggesting a possible role for NPR1 in the perception of not only SA but also NHP.

SA-MEDIATED IMMUNITY REQUIRES NPR1

To elucidate the SA-signaling pathway, multiple genetic screens have been performed in *Arabidopsis*, which led to the identification of the *NPR1* gene as a positive regulator (Figure 1). The *npr1* mutants display increased disease susceptibility and insensitivity to SA in the induction of defense genes and SAR.[11–14] Conversely, overexpressing *Arabidopsis* NPR1 (*AtNPR1*) leads to enhanced disease resistance to a wide range of pathogens in diverse plant species,[15,17–28,63,64] demonstrating NPR1's potential as a tool for engineering broad-spectrum disease resistance in agriculture.

However, the road to this ultimate goal has been a long one with many obstacles. Examining the NPR1 protein domains did not inform much about its molecular function (Figure 2).[65,66] The presence of 17 cysteines (of which 10 are conserved among all NPR1-like proteins)[67] suggested that the NPR1 protein might be sensitive to cellular redox changes, which were later shown to regulate the release of the protein from its quiescent oligomeric state. The presence of the N-terminal broad-complex, tramtrack, and Bric-à-brac (BTB) domain in NPR1 led to the hypothesis that it might serve as a substrate adaptor for cullin3-ring E3 ligase (CRL3), similar to other BTB-domain-containing proteins,[68] in order to degrade a repressor(s) of SAR; however, this repressor has remained elusive. The presence of a nuclear localization signal (nls) in the C terminus of NPR1 is required for its SA-induced translocation into the nucleus. However, moving the protein into the nucleus is not sufficient for the induction of defense genes,[69] implying that there are additional activation steps for NPR1 in the nucleus. The absence of a DNA-binding domain led to the hypothesis that NPR1 is a transcriptional cofactor functioning through association with a TF such as TGA (TGACG-binding TF).[70,71] However, binding of TGA to its *cis*-element is independent of NPR1,[72] raising the question of how NPR1 activates TGA to reprogram transcription in response to SA induction. The biggest puzzle for the field has been NPR1's relationship with SA. Although the phenotype of the *npr1* mutants suggests that the WT NPR1 is an SA receptor, the SA-binding affinity of NPR1 is significantly lower than that of its paralogs NPR3 and NPR4,[73,74] which are negative regulators of NPR1-target genes.[75] Despite reports showing that the affinity of NPR1 to SA is sufficiently high for its function,[54,76] experimental data on how SA binding actually regulates its transcription cofactor activity were inconclusive.

The absence of a basic understanding of NPR1's molecular function and regulation makes it difficult and even risky to use it for engineering disease resistance in agriculture. For example, NPR1 regulates the crosstalk between SA and the growth hormones auxin[77] and gibberellin[78] as well as the defense hormones jasmonic acid (JA) and ethylene (ET) involved in inducing resistance to necrotrophic pathogens and insects[79–81] (Figure 1). Therefore, it is possible that activation of SA-mediated

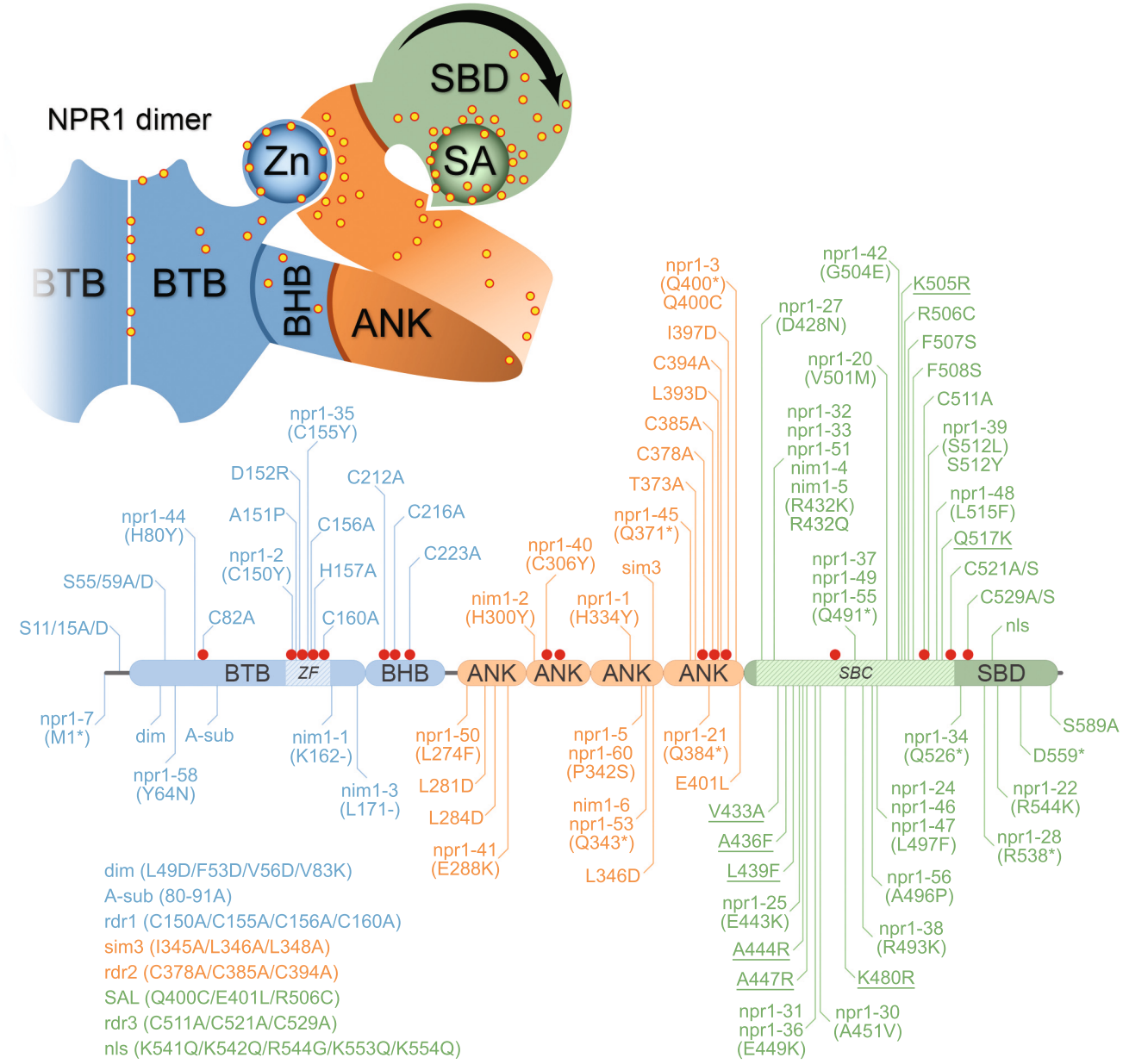

Figure 2. Map of mutations in *Arabidopsis* NPR1 generated through forward and reverse genetic approaches

Top, a spatial model of the partial NPR1 dimer. Bottom, a linear model of the NPR1 monomer. Locations of point mutations are mapped onto the spatial model as red-lined yellow dots and detailed on the linear model. Multiple mutations are dim, dimerization; A-sub, alanine substitution; sim3, SUMO-interacting motif 3; rdr1/2/3, redox-associated disorder region 1/2/3; nls, nuclear localization signal; SAL, SBD-ANK locked; SBC, SA-binding core. Underlined mutations correspond to those identified in *Arabidopsis* NPR4. Asterisks indicate STOP codon. Red dots on the linear model indicate positions of cysteine residues.

resistance against biotrophic and hemibiotrophic pathogens through NPR1 overexpression may lead to decreased plant growth and increased susceptibility to necrotrophic pathogens and insects.

Fortunately, as detailed below, some of the obstacles mentioned above have recently been overcome by new advances made in NPR1's structure-and-function studies, discoveries of the regulatory mechanisms of NPR1 activities, and also the proof-of-concept application of new molecular switches in controlling NPR1 protein accumulation in rice.[82]

NPR1 REPROGRAMS TRANSCRIPTION BY FORMING AN ENHANCEOSOME

The effects of SA on NPR1 were initially studied using the yeast one-hybrid assay where the C terminus of NPR1 was found to

harbor a transactivation domain that is sensitive to SA.[83] Subsequent studies established that NPR1-mediated transcription can be inhibited through its C-terminal association with NIM1(NPR1)-interacting proteins (NIMINs), which are repressors carrying a conserved ET-responsive-element-binding-factor-associated amphiphilic repression "EAR" motif.[83–85] SA can relieve this inhibition by disrupting NPR1's interaction with NIMIN through a conformational change to obscure the NIMIN-binding motif.[83,86] A single amino acid change in the motif, *nim1-4* (R432K),[66] severely impairs NPR1's ability to activate SAR. Maier et al.[83] found that this mutation rendered the interaction between nim1-4 with NIMIN-1 or NIMIN-2 non-responsive to SA in both *Arabidopsis* and tobacco. However, the expression of *NIMINs* is dependent on SA and NPR1,[85] suggesting that this SA-induced disruption of the NPR1-NIMIN interaction is unlikely the mechanism by which SA initiates NPR1-mediated transcriptional reprogramming.

A major breakthrough in understanding NPR1 transcriptional cofactor activity came from recent structural studies of NPR1 and its paralog NPR4.[73,87] Cryoelectron microscopy (cryo-EM) and subsequent mutagenesis analyses showed that the active NPR1 is a homodimer.[87] Moreover, X-ray crystallography of the BTB domain revealed a distinct C_2HC type zinc finger (Figure 1), which plays an essential role in the structural integrity of NPR1 enabling its interaction with the TGA TF and oligomerization.[87] In the NPR1-TGA3 complex, each monomer of the NPR1 dimer interacts with a TGA3 TF dimer (i.e., $TGA3_2$-$NPR1_2$-$TGA3_2$). Analysis of the top 100 SA-induced gene promoters found that 77 of them contained at least two TGA-binding *as-1* elements. Indeed, gel mobility shift assay showed that NPR1 caused a "supershift" of the TGA3-DNA band only when both *as-1* elements were bound by TGAs.[87] This indicates that NPR1 induces defense gene transcription by bridging the DNA-bound TGA TFs to form an enhanceosome that brings together *as-1* elements present either on the same or different gene promoters, possibly through DNA looping.

With regard to the effect of SA on NPR1 transcriptional activity, the answer came from the structural study of the NPR1-paralog NPR4, which has a much higher SA-binding activity, showing that the C-terminal region of NPR4 contains the SA-binding domain (SBD).[73] Surprisingly, the residues involved in SA binding in NPR4 are conserved in NPR1, including R432 identified in the *nim1-4* mutant[66] (Figure 2). The difference in their SA-binding activities was explained by the distinct residues in the SBDs of NPR1 and NPR4.[73] In the absence of SA, the SBD domain of NPR1 was found to be disordered.[87] SA induces the folding of SBD, which then docks onto the ankyrin repeat (ANK) domain. Mutating the residues at the SBD-ANK interface (Figure 2) abolished NPR1 transcriptional cofactor activity. Conversely, introducing two proximal cysteine residues at the interface to lock SBD and ANK in the docked conformation enhanced SA-induced target gene expression, providing the first structural evidence for a direct role of SA in controlling NPR1 transcriptional activity.[87] This result also suggests possibilities for designing more active NPR1 variants (Figure 2).

The NPR1 enhanceosome is likely to contain other proteins besides TGAs. In the cryo-EM samples, besides the hexameric $TGA3_2$-$NPR1_2$-$TGA3_2$, the tetrameric $NPR1_2$-$TGA3_2$ intermediates were also detected, suggesting that NPR1 may form a complex with other TFs either separately or with a TGA dimer on one side and another TF on the other side. Indeed, in the presence of both SA and JA, NPR1 has been found to interact with MYC TFs to inhibit JA-responsive gene transcription as an SA-mediated crosstalk mechanism.[88] WRKYs are another family of TFs that have been shown to interact with NPR1.[89] Consistent with their role in SAR, the *cis*-element for WRKYs, the W-box, is the most enriched promoter element found in the induced genes in all the SA-related transcriptomic data.[54,90,91] In fact, some of the *WRKY* genes, particularly those encoding members of group III WRKYs, are NPR1 induced.[90,92] Curiously, only the transcriptionally inactive unsumoylated NPR1 was found to interact with WRKY70, which was shown to inhibit expression of SA synthesis genes,[90] suggesting that NPR1 may first remove WRKY70 repression on *ICS1* expression before activating SA-responsive genes.[89,90] However, fluorescence imaging showed that NPR1 and WRKY70 interaction mainly occurs in the cytoplasm instead of in the nucleus,[8] leaving the question of which WRKY TF(s) is responsible for the transcriptional reprogramming through NPR1 unanswered.

Besides TFs, the NPR1 enhanceosome is likely to recruit large transcription regulatory machineries because upon SA induction, NPR1-GFP can be observed as nuclear condensates.[8,89] It is plausible that these condensates contain the Mediator complex because several of its components have been shown to be required for SA-mediated gene expression and resistance.[93] Also, histone acetyltransferases (HACs) were found to form a HAC-NPR1-TGA complex and be required for activation of a subset of SA-induced genes.[94] Besides these components, a comprehensive survey of the NPR1-enhanceosome is required to identify all the players involved in SA/NPR1-mediated transcriptional reprogramming. Whether they are similar to those recently identified in the SA-induced guanylate-binding-protein-like GTPase (GBPL) transcriptional condensates[93,95,96] remains to be determined.

NPR1-INDUCED DEFENSE PROTEIN HOMEOSTASIS

The SA/NPR1 signaling pathway induces not only a large number of "*PR* genes" encoding secreted anti-microbial peptides but also many "*ER* genes" encoding ER-resident secretion and folding machinery proteins to protect the cells from an overload of the defense proteome[10] (Figure 1). Interestingly, analysis of these *ER* gene promoters identified a conserved *cis*-element CTGAAGAAGAA named "TL1," which was later found to be targeted by a heat shock factor-like TL1-binding factor 1, TBF1. The *tbf1* mutants have unaltered *PR1* gene transcript levels, yet significantly less protein secretion. Conversely, overexpression of the *TBF1*-coding sequence results in plant cell death.[82,97] Even more interestingly, the level of TBF1 appeared to be tightly regulated at not only the transcriptional level but also the translational level. TBF1 protein translation is normally inhibited by the two upstream open reading frames (uORFs) in the 5′ leader sequence (5′ LS) of its mRNA. The inhibition is rapidly and transiently alleviated upon pathogen challenge.[82,97] This finding demonstrates that maintaining defense protein homeostasis is of life-and-death importance for plants, and one major challenge

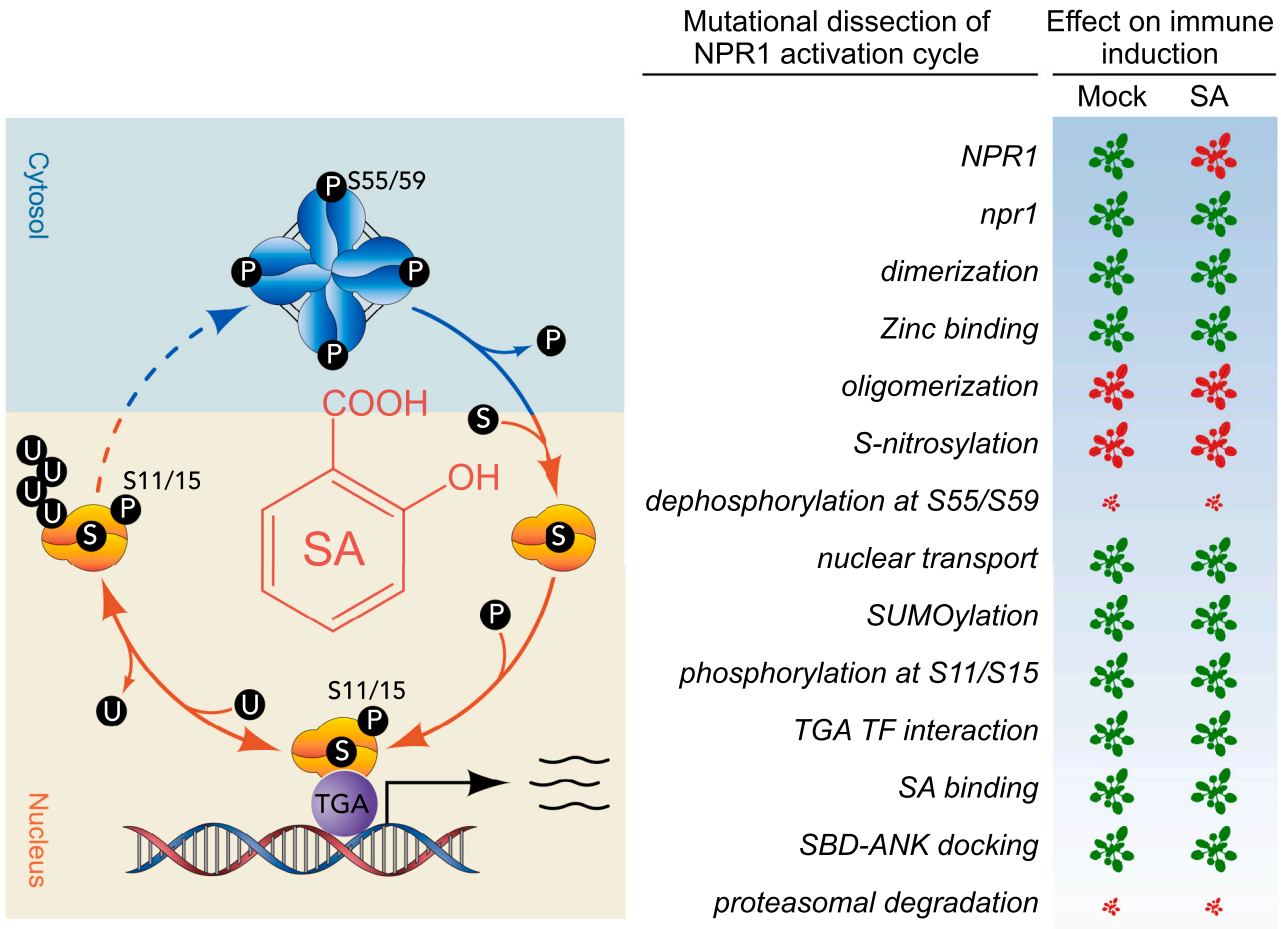

Figure 3. Activation cycle of NPR1

Left: SA-initiated PTMs of NPR1 and activation of defense transcription. P, phosphorylation; S, SUMOylation; U, ubiquitination. Right: mutations of PTMs of NPR1 and their effect on plant immunity under basal (mock) and SA-induced conditions. Green plants indicate a lack of immune induction; red plants indicate induced immunity; small red plants indicate autoimmunity with retarded plant growth.

in engineering disease resistance is not how to turn on defense but rather how to precisely mount a defense response without harming self. Conservation of TBF1 in not only the coding sequence but also the 5′ LS[82] indicates that plants have evolved mechanisms to manage the expression of dangerous, but important, defense proteins. How understanding of these mechanisms can lead to the development of new engineering strategies will be discussed below.

In addition to coordinately regulating both the antimicrobial *PR* genes and the *ER* genes to ensure proper deployment of the defense proteome, a new role for NPR1 in regulating defense protein homeostasis was serendipitously discovered through a cellular study of a possible role for NPR1 as a substrate adaptor for CRL3.[8] Fractionation experiments showed that NPR1 is required for SA-induced protein ubiquitination in the cytoplasm by forming biomolecular condensates (cSINCs). These condensates are enriched with stress response proteins, including multiple NB-LRR immune receptors and their downstream signaling components such as enhanced disease susceptibility 1 (EDS1) required for ETI-mediated PCD, oxidative and DNA damage-response proteins, and protein quality control machineries. Consistent with NPR1's role in promoting cell survival,[8,98] cSINCs were observed in naive cells adjacent to those undergoing ETI-mediated PCD (Figure 1). In support of the hypothesis that NPR1 serves as a CLR3 adaptor to control stress protein homeostasis, the transition of NPR1 into condensates triggers the formation of the NPR1-Cullin3 complex in order to ubiquitinate cSINC-localized substrates, such as EDS1, and specific WRKY TFs required for ETI.[8] Moreover, NPR1 also promotes cell survival against heat shock, UV irradiation, and oxidative damage,[8] indicating that NPR1-induced defense operates at the level of maintaining cellular homeostasis in response to both biotic and abiotic stresses. Interestingly, mutating the redox-sensitive disorder region 3 in NPR1 (Figure 2) abolished the protein's ability to form both nuclear and cytoplasmic condensates, although they are expected to have different components and biological functions. The intrinsic ability of NPR1 in organizing these biomolecular condensates to reprogram transcription and control stress protein homeostasis provides an explanation of how this single protein can have such a wide range of protective activities.

REGULATION OF NPR1 ACTIVITY

The distinct functions of NPR1 are regulated by post-translational modifications (PTMs) (Figure 3). The nuclear localization of NPR1 is necessary, but not sufficient, for the expression of *PR* genes.[67,69,72] In the resting state, NPR1 is present in the cytoplasm as an oligomer.[67,69,83,99–102] Accumulation of SA and associated transient oxidative burst trigger a compensatory increase in the reduction power of the cell to release NPR1 from the oligomer to translocate into the nucleus.[67] S-nitrosylation of Cys156 facilitates the formation of the quiescent NPR1 oligomer, whereas thioredoxins, particularly thioredoxin H-type 3 (TRX-h3) and thioredoxin H-type 5 (TRX-h5), reduce Cys156 and partially disassemble the oligomer[67,103] (Figure 3). Recent analysis showed that the reduced form of NPR1 is not a monomer but rather a dimer.[87] Inhibiting dimer formation abolishes NPR1's transcriptional activity,[87] whereas mutating C82 and C216 at the dimer and oligomer interfaces, respectively, increases the basal expression of defense genes (Figure 3).[67,69,103] After being released from the oligomer, NPR1 requires multiple PTMs to be activated, besides binding to SA[87] (Figure 1). Dephosphorylation of NPR1 at S55/S59 is required for its interaction with SUMO3 and SUMOylation, which, in turn, is required for NPR1's nuclear retention and interaction with TGA TF[89] (Figure 3). SUMOylation is also a prerequisite for phosphorylation at S11/S15 and subsequent CRL3-mediated ubiquitination and turnover of NPR1.[104] Interestingly, proteasome-mediated nuclear turnover of NPR1 facilitates, instead of inhibits, the induction of SA-responsive genes[104] (Figure 3). This is governed by a "ubiquitination relay" mechanism, which starts with CRL3-mediated mono-ubiquitination that activates NPR1 to induce transcription, followed by poly-ubiquitination by UBE4 and de-ubiquitination by UBP6/7 that degrade and stabilize NPR1, respectively.[105] Phosphatases and kinases responsible for NPR1 phospho-regulation have yet to be identified. Whether an NPR1-modifying enzyme can be a good target for engineering disease resistance is still unknown.

PATHOGEN-TRIGGERED EXPRESSION OF NPR1 FOR ENGINEERING RESISTANCE WITHOUT FITNESS COSTS

Immune responses are known to slow plant growth, and, conversely, active growth and development are associated with reduced defense.[106,107] The success in enhancing broad-spectrum disease resistance in various crop species through the overexpression of *AtNPR1*[15–32] suggests that the level or activity of the endogenous *NPR1*s in these plants is not optimal, possibly due to domestication that favored higher biomass over pathogen resistance. It also suggests a high degree of functional conservation not only in NPR1 itself but also in the components of the SA/NPR1-signaling pathway in plants.

Although *AtNPR1* overexpression has led to a significant improvement in disease resistance and, even in yield, without a detectable growth phenotype in some crops,[18,19,21,23,24,26,29,32,108–111] the negative effects on growth, yield, or insect resistance have been reported in rice, wheat, and strawberry.[111–114] These fitness costs can be alleviated by expressing *AtNPR1* only where pathogens proliferate, such as in green tissues to protect rice against sheath blight disease,[28] and in the phloem to protect orange trees against citrus greening disease[27] (Figure 4). Thus, a more localized and possibly temporally controlled expression of *AtNPR1* would be preferable over global expression to avoid the negative effects on growth and defense against necrotrophic pathogens/insects due to SA/NPR1-mediated crosstalk with JA and ET.

The low copy number of *NPR1* orthologs in a sample of angiosperm genomes[115] indicates not only their functional conservation across lineages but also a tight control of NPR1 dosage in plants. Overexpression of NPR1 orthologs from apple,[20,116–118] strawberry,[119] mulberry,[120] grapevine,[25] cabbage,[21] kiwifruit,[121] citrus,[122] *Brassica napus*,[123] gladiolus,[124] lily,[125] rye,[126] and wheat[127] enhanced disease resistance not only in the respective plant species but also in heterologous backgrounds. However, when the rice *NPR1* (*OsNPR1/NH1*) was overexpressed in *Arabidopsis*, its complementation of the *npr1* mutation in resistance against biotrophic pathogens was accompanied by enhanced susceptibility to insects.[22]

From overexpressing *NPR1*, a great leap forward in engineering broad-spectrum disease resistance came from studies of the pathogen-inducible expression of the TBF1 TF.[82] Placing *AtNPR1* under the control of the *TBF1* promoter and 5′ LS made expression of the gene pathogen-inducible at both transcriptional and translational levels, respectively. Transgenic rice with such controlled NPR1 expression showed enhanced resistance to rice blast, rice blight, and bacterial leaf streak diseases without yield penalties in the field[82] (Figure 4). This provided conceptual proof that it is possible to engineer broad-spectrum disease resistance for agricultural applications by making immune activation transient. Such a strategy can significantly reduce fitness costs commonly associated with sustained activation of the immune response. Moreover, transient pathogen-triggered activation of the SA/NPR1 pathway can reduce the crosstalk between SA and other defense hormones to avoid interfering with resistance against necrotrophic pathogens and insects. Broad-spectrum resistance is also a possible solution for controlling emerging diseases, for which no specific resistance genes (e.g., NB-LRR) have evolved.

PERSPECTIVES

There are still many unknowns in the functioning of NPR1 that remain to be resolved. These include the following: what is the missing factor that enables NPR1 to bind to SA with high affinity in planta? Is NPR1 also involved in the perception of NHP which has been shown to function synergistically with SA through the activity of NPR1? What are the conditions that control SA-induced NPR1 condensate formation in the cytoplasm and the nucleus? What are the specific components of the NPR1 enhanceosome that enable fine-tuning of transcriptional reprogramming to meet conditions of a particular pathogen/stress? How does the NPR1 signaling pathway coexist with other hormonal pathways to optimize plant responses under composite stress conditions? And what are the determinants of NPR1 regulation in abiotic stress, such as salinity, drought, and cold (Figure 1)?

Despite its central role in plant health, reports implicating NPR1 as a target of pathogen effectors are scarce. One

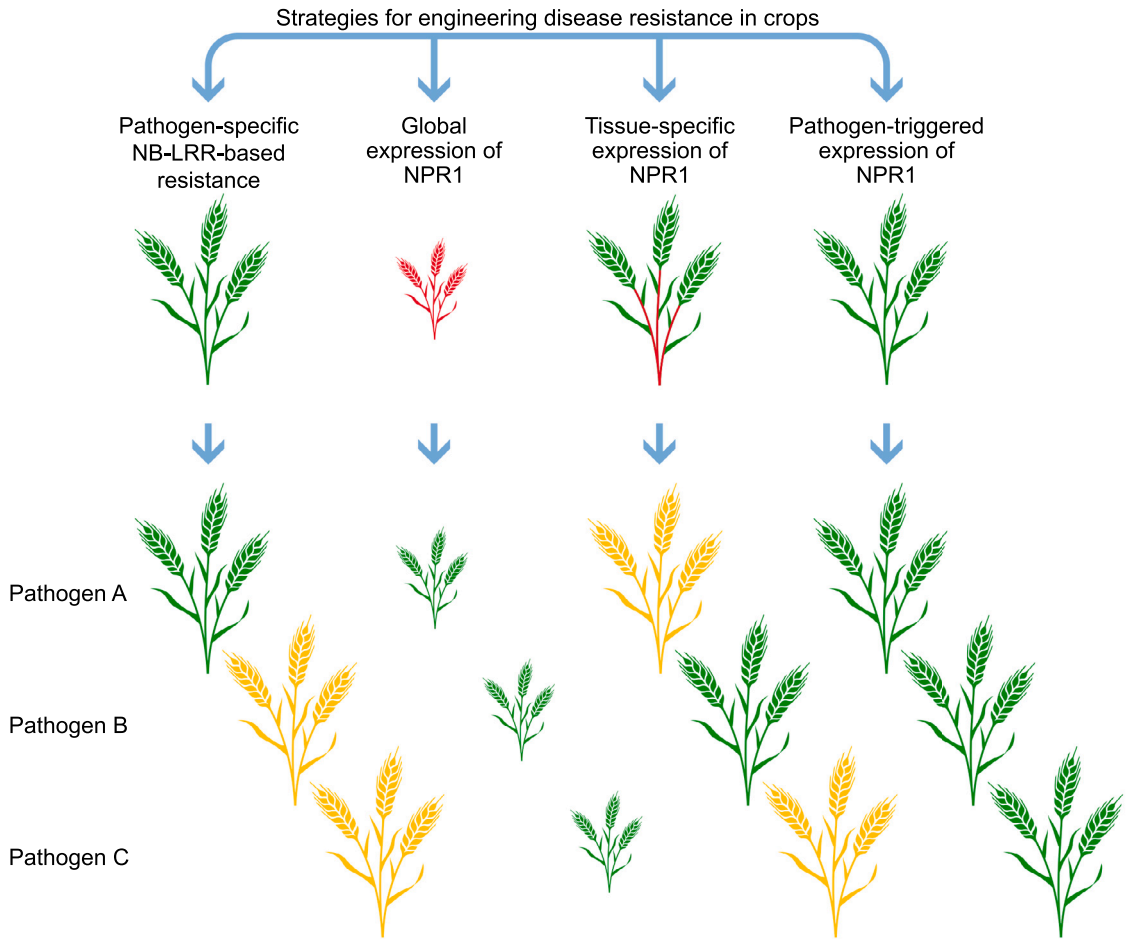

Figure 4. Strategies for engineering pathogen-specific and broad-spectrum (with NPR1 as an example) disease resistance in crops
In the upper panel, green indicates plants with un-induced immunity, and red indicates plants with elevated immunity. In the lower panel, green indicates plants with resistance, and yellow indicates plants with disease development. The smaller sized plants indicate fitness penalty as a result of constitutive immunity.

study found a bacterial effector interacting with NPR1 in *Arabidopsis*[128] and another showed a fungal effector targeting NPR1 in wheat,[129] both of which led to suppressed immunity against the respective pathogens. However, in principle, NPR1 is an unlikely target for pathogens because in unchallenged plants, NPR1 is locked in the inactive oligomeric form. More importantly, during infection, NPR1 is fully activated and functional in the uninfected systemic cells yet to be challenged by a pathogen. In fact, NPR1 has to be degraded in the infected tissue to allow ETI to proceed,[74] and activation of NPR1 prior to infection fully blocks this local immune response.[8]

Rapid development of CRISPR-Cas9-based genome-editing tools[130] will allow the introduction of the wealth of AtNPR1 variants with a wide range of activity levels into different genetic backgrounds (Figures 2 and 3). Moreover, altering the endogenous *NPR1* orthologs in crops by changing their expression level, tissue distribution, and timing of expression will facilitate the engineering of optimal resistance with minimum fitness costs. A crucial step in engineering effective disease resistance is to provide plants with the ability to sense the pathogen and mount a defense only when and where it is needed. Therefore, combining the above genomic approaches with novel molecular switches, such as those involved in controlling pathogen-induced translation,[82,131,132] will revolutionize the design of disease resistance in crops using immune regulators like NPR1 (Figure 4).

ACKNOWLEDGMENTS

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DECLARATION OF INTERESTS

X.D. is a founder of Upstream Biotechnology Inc. and a member of its scientific advisory board, as well as a scientific advisory board member of Inari Agriculture Inc. and Aferna Bio.

List of patent applications:

R.Z. and X.D. have patents related to Enhanced cell survival against biotic and abiotic stresses through salicylic acid-induced NPR1 condensates, WO2021262685A2 (pending), and R.Z., P.Z., and X.D. have patents related to NPR1 variant to enhance plant resistance to biotic and abiotic stresses and method thereof. US Provisional Patent Application, #63/463338 (pending).

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