Review

Tell me more: roles of NPRs in plant immunity

Karolina M. Pajerowska-Mukhtar, David K. Emerine, and M. Shahid Mukhtar

Department of Biology, University of Alabama at Birmingham, Birmingham, AL 35294-1170, USA

Plants and animals maintain evolutionarily conserved innate immune systems that give rise to durable resistances. Systemic acquired resistance (SAR) confers plant-wide immunity towards a broad spectrum of pathogens. Numerous studies have revealed that NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR) is a key regulator of SAR. Here, we review the mechanisms of NPR1 action in concert with its paralogues NPR3 and NPR4 and other SAR players. We provide insights into the mechanisms of salicylic acid (SA) perception. We discuss the binding of NPR3 and NPR4 with SA that modulates NPR1 coactivator capacity, leading to diverse immune outputs. Finally, we highlight the function of NPR1 as a *bona fide* SA receptor and propose a possible model of SA perception *in planta*.

BackStory – plant immune systems and systemic acquired resistance

Hosts and their potential pathogens are engaged in a constant coevolutionary battle for dominance. For protection against infections caused by invading microorganisms, plants and animals have evolved highly complex and effective immune systems [1]. The mechanisms of the immune system are under constant selective pressures as hosts continuously refine their ability to recognise and respond to ever-changing pathogenic virulence strategies while preserving tolerance to self-antigens [1]. Although plants and animals are separated by over one billion years of evolution and exhibit fundamental differences in their immune systems, they share diverse germline-encoded receptors capable of non-self recognition. Over the past 500 million years, jawed vertebrates have evolved highly orchestrated innate and adaptive immune systems for protection against microbial attacks [2]. Mammalian immune responses include pathogen recognition, creation of antigen-specific receptors, and immunological memory cells that circulate in the blood [3]. By contrast, plants solely rely on the capacity of each individual cell to recognise and mount defence responses against a wide range of pathogens, such as viruses, bacteria, fungi, oömycetes, and nematodes [4].

Plants are able to recognise pathogens using two major classes of receptor. Initially, microbes are detected via perception of conserved microbial-associated molecular patterns (MAMPs) by plant pattern-recognition receptors (PRRs) located on the cell surface. This first level of recognition results in MAMP-triggered immunity (MTI), which is sufficient to fend off most invading microbes [5]. To circumvent MTI, phytopathogens secrete and deliver effector proteins into host cells [6,7]. Recently, it was shown that independently evolved pathogen effectors converge on a limited set of highly interconnected host proteins that play crucial roles in plant immunity [8]. As a counter defence, plants deploy a second set of polymorphic intracellular immune receptors that recognise specific effectors. Nearly all of the polymorphic intracellular immune receptors are members of the nucleotide binding site-leucine rich repeat (NB-LRR) protein family, analogous to the animal innate immune nucleotide-binding domain and leucine-rich repeat-containing receptor (NLR) proteins [9,10]. NB-LRR activation causes effector-triggered immunity (ETI), which resembles an escalated MTI response and leads to robust disease resistance that often includes localised host cell death or a hypersensitive response (HR) [6,11]. Stimulation of defence responses occurs not only at the site of pathogen recognition but also in distal regions of the plant, a phenomenon known as systemic acquired resistance (SAR) [12–15]. SAR is an effective innate immune response that provides protection against a broad range of biotrophic pathogens. SAR can also be induced by treating plants with salicylic acid (SA) or its biologically active analogues 2,6-dichloroisonicotinic acid (INA) and benzothiodiazole (BTH) [12–15]. Since A. Frank Ross published the first report of SAR in a tobacco (*Nicotiana tabacum*)–tobacco mosaic virus pathosystem over half a century ago [16], research has primarily focused on understanding how plants mount long-distance resistance in the absence of classical adaptive immunity and circulatory systems. SAR is thought to be a consequence of the concerted action of massive ETI- and/or MTI-triggered transcriptional changes, increased cellular concentration of SA, the activation of multiple downstream signalling cascades, and the production of antimicrobial peptides such as pathogenesis-related (PR) proteins [17,18]. In the model plant *Arabidopsis thaliana*, SA signals through the central immune regulator NPR1, also known as NON-INDUCIBLE IMMUNITY 1 (NIM1), SALICYLIC ACID INSENSITIVE 1 (SAI1), or ENHANCED DISEASE SUSCEPTIBILITY 17 (EDS17) [13,19–21].

NPR1 has been implicated in growth, development and diverse immune signalling pathways such as basal defence, SAR, induced systemic resistance, and ETI, as well as in mediating crosstalk between SA and other

Corresponding author: Mukhtar, M.S. (smukhtar@uab.edu)

Keywords: NPR1; NPR3; NPR4; salicylic acid receptor; systemic acquired resistance; proteasome-mediated degradation.

1360-1385/$ – see front matter.
Published by Elsevier Ltd. http://dx.doi.org/10.1016/j.tplants.2013.04.004

phytohormones [13,22–28]; however, in this review we focus on the latest breakthroughs in our understanding of SA perception by NPR1 and its paralogues NPR3 and NPR4 [29–31]. Furthermore, the mechanisms of proteasome-mediated NPR1 degradation are discussed to understand how NPR1-instigated signals are precisely deciphered to trigger specific responses responsible for making cellular fate decisions [29,32]. Detailed functions of the additional NPR1 paralogues NPR2, NPR5, and NPR6 are beyond the scope of this review.

First listen – current understanding of NPR1 in SAR

Studies over the past two decades have revealed that NPR1 is a key transcriptional regulator that controls a wide range of host genes involved in various immune responses and organismal fitness [17,24,33]. Structurally, NPR1 contains two conserved protein–protein interaction motifs: an ankyrin repeat domain and a broad complex, tramtrack, and bric-à-brac/poxvirus and zinc-finger (BTB/POZ) domain [34]. NPR1 shares structural features with mammalian immune cofactor IκB, which controls the activity of nuclear factor kappa B (NF-κB) in the cytoplasm and nucleus [35]. Intriguingly, mustard (*Brassica juncea*) NPR1 was shown to inhibit NF-κB activation, suggesting functional conservation of key immune players across kingdoms [34–36]. Plants lacking functional NPR1 are impaired in their ability to express *PR* genes and are almost completely defective in mounting SAR in response to pathogen infection. Biochemical and genetic studies, in particular with *Arabidopsis*, have revealed finely tuned regulation of NPR1 in the cytoplasm and nucleus to establish effective SAR [14]. However, several conflicting reports exist on how NPR1 is regulated in both naïve and infected cells. According to one model, NPR1 is predominantly sequestered in the cytoplasm of unstimulated cells as a high molecular weight oligomeric complex that is stabilised through intermolecular disulfide bonds between conserved cysteines [37,38] (Figure 1A). Increased cellular SA concentration as a result of pathogen infection elicits thioredoxin-mediated reduction of cysteine 156 (Cys156) and releases monomeric NPR1. This monomeric form of NPR1 is translocated to the nucleus via a C-terminal bipartite nuclear localisation signal (NLS), where it exerts regulatory activities, including *PR1* gene

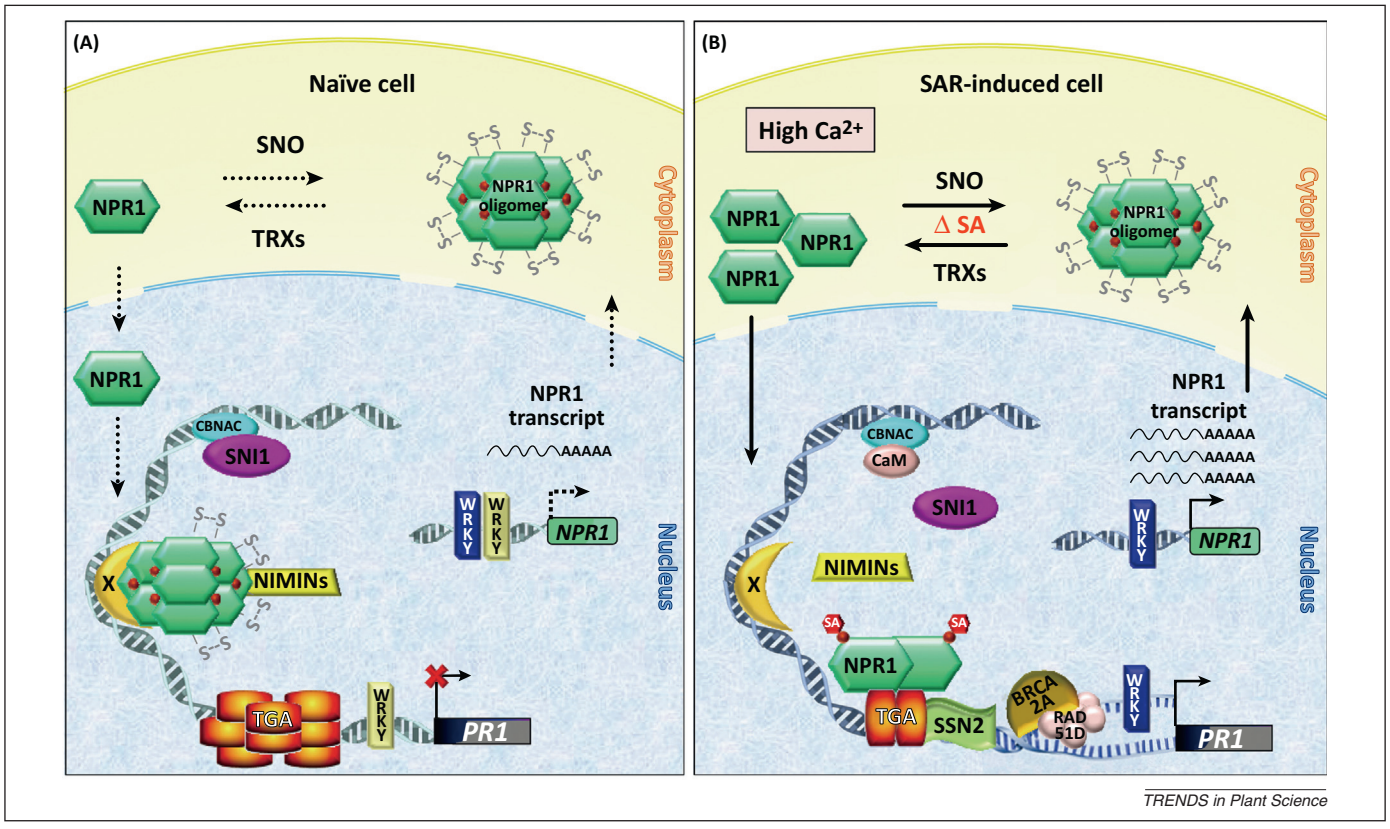

Figure 1. A model for the NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1)-mediated salicylic acid (SA) signalling pathway. (**A**) In uninfected cells, large amounts of inactive oligomeric NPR1 containing transition metal atoms (bright-red solid circles) can be present in both the cytoplasm and the nucleus. Oligomerisation and monomerisation of NPR1 can be facilitated by *S*-nitrosothiol (SNO) and thioredoxins (TRXs), respectively. In the absence of stimuli or pathogen infection, the kinetics of NPR1 monomerisation are relatively low and a minute amount of the active NPR1 monomer can be translocated to the nucleus. In the naïve cell, oligomeric NPR1 interacts with the repressor protein NON-INDUCIBLE IMMUNITY 1 (NIM1)-INTERACTING (NIMIN). This NPR1/NIMIN complex can be found on PATHOGENESIS-RELATED GENE 1 (*PR1*) upstream regulatory sequences. In addition, SUPPRESSOR OF NPR1 INDUCIBLE 1 (SNI1) also represses *PR1* gene expression, most likely by interacting directly with CALMODULIN-REGULATED NAC TRANSCRIPTION FACTOR (CBNAC), which has an affinity to bind with the *PR1* promoter. Inactive octameric TGA and repressive WRKY transcription factors also suppress *PR1* transcription. A combination of both activating and repressive WRKY factors fine-tunes the regulation of *NPR1* transcription and maintains NPR1 homeostasis in the absence of pathogen infection. (**B**) In systemic acquired resistance (SAR)-induced cells, increased concentrations of SA allow the translocation of relatively large quantities of active NPR1 monomer into the nucleus. Two molecules of SA bind directly with NPR1 dimer via transition metal atoms. Active forms of both NPR1 dimer and a TGA dimer interact physically on the *PR1* promoter. Additional SAR signalling components, such as SUPPRESSOR OF SNI1 2 (SSN2), a SWI2/SNF2 domain-containing protein, RADIATION SENSITIVE 51D (RAD51D), BREAST CANCER 2A (BRCA2A), and activating WRKY transcription factors are recruited onto the *PR1* promoter. The repressor proteins, including SNI1, NIMINs, and repressive WRKY factors, are dissociated from the *PR1* promoter. All of these events subsequently lead to the induction of *PR1* gene expression and immune responses.