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NRC proteins - a critical node for pattern and effector mediated signaling

Meirav Leibman-Markus[a], Lorena Pizarro[a], Maya Bar [b], Gitta Coaker[c], and Adi Avni [a]

[a]School of Plant Sciences and Food Security, Tel Aviv University, Tel Aviv, Israel; [b]Department of Plant Pathology and Weed Research, ARO, Volcani Center, Rishon LeZion, Israel; [c]Department of Plant Pathology, University of California, Davis, California, USA

ABSTRACT

Plants are constantly exposed to numerous diverse microbes and pests. They lack an adaptive immune system and rely on innate immunity to perceive and ward off potential pathogens. The plant immune system enables plants to overcome invading microorganisms, and can be defined as highly successful in this regard. Nevertheless, specialized pathogens are able to overcome structural barriers, preformed defenses, innate immunity and are a persistent threat to crop and food supplies worldwide. The rapidly growing world population results in massive demands for agricultural products and reliable crop yields. Therefore, the ability to precisely manipulate plant immunity to resist diverse diseases holds significant promise for enhancing crop production.

Plant immunity is conventionally depicted as a two-tiered system. The first line of plant defense is formed by pattern recognition receptors (PRRs), located at the cell surface, that recognize microbe-associated molecular patterns (MAMPs) and trigger pattern-triggered immunity (PTI).[1] Successful pathogens are able to overcome plant PTI using secreted effectors that suppress PTI, leading to plant susceptibility. Concomitantly, plants evolved the second line of defense based on cytoplasmic immune receptors that recognize effectors (Avrs) and deploy effector-triggered immunity (ETI).[2,3] However, accumulating evidence from the last decade blurs the distinction between the two systems and reveals significant overlap and diversity in immune signaling networks.[4–6]

Blurred distinction

MAMPs are traditionally defined as highly conserved moieties within a class of microbes, essential for microbial fitness,[7,8] while effectors are considered to be species, race or strain specific and confer virulence.[9,10] However, several MAMPs and effectors refuse to follow these rules, creating exceptions to this man-made distinction. For instance, the necrosis-induced peptide 1 (NEP1) and NEP1-like proteins are effectors required for virulence, but they are conserved among bacteria, fungi and oomycetes.[4,5] Other examples of widespread effectors required for pathogen virulence are extracellular protein 6 (Ecp6) and Ecp6 conserved orthologs – LysM effectors, harpin effectors and crinkler effectors that are widely conserved in fungi, Gram-negative bacteria and oomycetes, respectively.[4,5] On the other hand, we find examples of MAMPs with a very narrow known distribution such as Ax21 and Pep-13 that are present in only a few *Xanthomonas* strains and *Phytophthora* species, respectively.[4,5] Additionally, MAMPs such as flagellin, lipopolysaccharide,

peptidoglycan, chitin, EIX and PWL (pathogenicity toward weeping lovegrass) contribute to pathogen virulence.[4,5]

The distinction between the outcomes of PTI and ETI can also be confusing. ETI is frequently noted as leading to a more rapid, sustainable and stronger immune response, culminating in cell death known as the hypersensitive response (HR).[2,11] Nonetheless, we know of several MAMPs that are able to induce HR, among them flagellin, CBEL and EIX.[12–15] Meanwhile, we can find weak ETI responses like the ones mediated by RPS4 and Ve1, which recognize *Pseudomonas* and *Verticillium* effectors, respectively.[16,17]

PTI is mediated by cell surface PRRs that can be classified, based on their domains, into different categories. The largest category is that of extracellular leucine-rich repeat (eLRR) containing receptors, and can be further divided into receptor like proteins and kinases (eLRR-RLP/K).[18] ETI is frequently mediated by cytoplasmic receptors, which are primarily nucleotide binding leucine rich repeat (NLR) proteins.[19] However, there are several examples of eLRR-RLP type cell surface receptors that can recognize effectors and mediate ETI, such as Cf2, Cf4, Hcr9-4E, Cf5, Cf9, Ve1, l and LepR3/RLM2,[20–27] and even an eLRR-RLK – I-3.[28] Given the gradient of responses that can be induced by either MAMP or effector recognition, it is now becoming clear that the responses governed by diverse plant immune receptors are integrated in diverse manners.[6]

Convergent immune signaling

Despite the diversity of pathogen components recognized and strength of immune responses, there are commonalities in defense signaling outputs, indicating that recognition of both MAMPs and effectors utilize similar signaling networks. PTI induces rapid activation of MAPK cascades which leads

to the activation of downstream signaling pathways.[29] Unsurprisingly, several effectors target MAPK cascade components in order to disrupt PTI.[30–34] More interestingly, there are cases of ETI leading to activation of the same PTI-MAPK cascades.[35] In addition, PTI/ETI share other signaling pathways including ROS signaling, Ca^{2+} signaling, hormone signaling and substantial overlapping transcriptional regulation, resulting in plant immune responses- as recently reviewed by Peng et al.[36] These evidences demonstrate a certain level of convergence in PTI/ETI signaling.

Helper NRCs as signaling convertors

In the classical gene-for-gene model proposed by Harold Flor in 1942, an *R* gene typically encodes an NLR receptor that detects and responds to an effector (*Avr*) gene product.[37,38] More recently, a diversity of recognition mechanisms have been revealed.[3] One additional model demonstrates that NLRs can function in pairs, with one NLR functioning as a sensor- for effector detection (s-NLR), and the other as a helper- initiating immune signaling (h-NLR),[39,40] (Figure 1,. One specific NLR family in *Solanaceae*, termed NLR required for cell death (NRC), emerges as a key family of h-NLR required signaling downstream of multiple s-NLRs.[41] Recent publications indicates NRCs function not only as h-NLRs mediating s-NLR signaling, but also as h-NLRs mediating MAMP and effector signaling sensed by diverse eLRR-receptors. NRC2

and NRC3 from *N. benthamiana* were shown to be involved in defense responses mediated by the eLRR-RLP Cf4, which recognizes the Avr4 effector from *Cladosporium fulvum*.[42] We have recently described tomato NRC4 as associated with an eLRR-RLP required for perception of a fungal MAMP – EIX (LeEIX2) and with an eLRR-RLK required for perception of bacterial flagellin (FLS2), enhancing defense responses mediated by them.[43] We have further shown that NRC4 is required for LeEIX2 and likely also FLS2-mediated defense responses. Furthermore, NRC4's N-terminal coiled-coil domain is sufficient to mediate the association with LeEIX2 and can enhance EIX and flagellin- elicited defense responses as efficiently as the full length protein.[43] Earlier works have indicated that tomato NRC1 may be involved in defense responses sensed by the eLRR-RLPs Cf4, Cf9, Ve1 and LeEIX2 as well.[44] Keeping with the sensor-helper model, the eLRR receptors – Cf4, Cf9, Ve1, LeEIX2 and FLS2 can be defined as sensors, detecting effectors and MAMPs. In this context, eLRR-RLP/K act as sensor (s-RLP/K), while NRCs keep their role as an h-NLR (Figure 1). **We propose that taken together, these data essentially position NRCs as a key signaling node required for the initiation of signaling sensed in both PTI and ETI immune pathways (Figure 1).**

In accordance with this concept, we have recently shown that DNA manipulation (using CRISPR-cas9 editing) of tomato NRC4 leads to significant enhancement of immunity. NRC4 CRISPRed plants, encoding a 67 aa truncated variant,
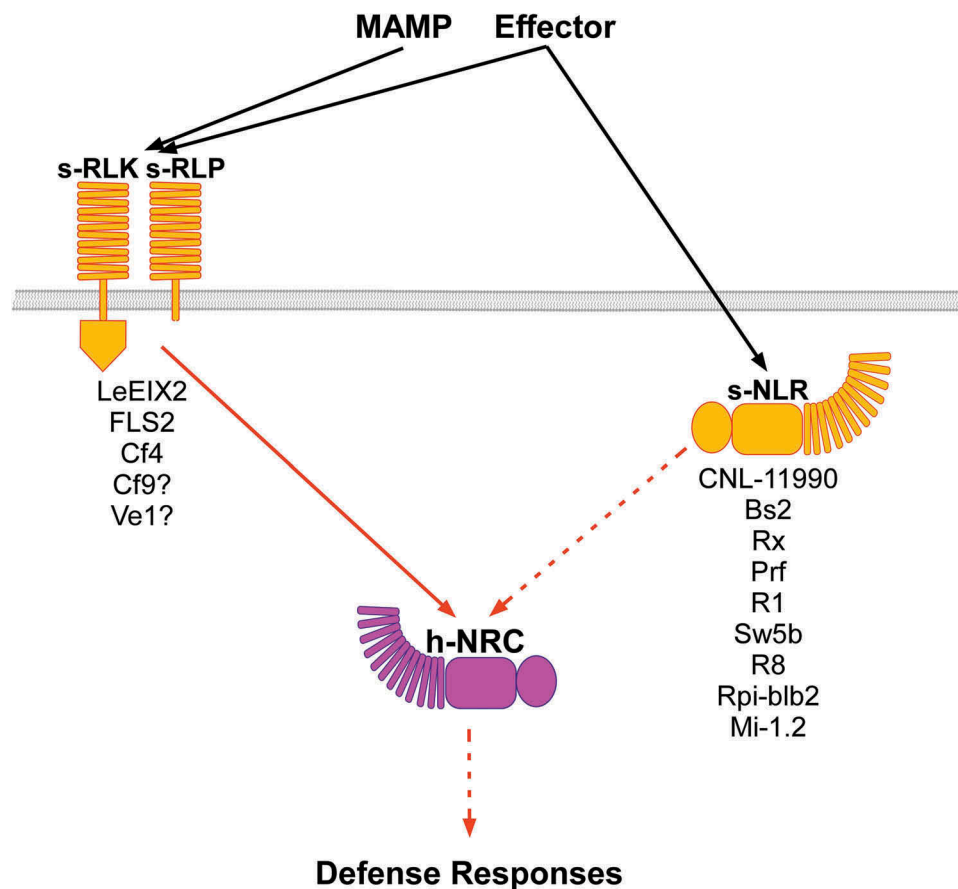
Figure 1. Schematic overview of plant defense signaling mediated by the NRC helper NLR (h-NRC). Perception of MAMPs or effectors by extracellular or intracellular immune receptors act as pathogen sensors (s-RLK, s-RLP, s-NLR) and lead to initiation of immune-signaling by a family of helper NLRs (h-NRC).

displayed intensified defense responses when challenged with EIX, and presented a higher resistance to *B.cinerea*.[43] Our work, together with previous publications,[41–44] positions NRC as a signaling funnel for multiple PTI and ETI sensor-receptors, demonstrating that CRISPR editing of NRCs could potentially result in agriculturally improved *Solanaceae* varieties possessing resistance to a broad spectrum of pathogens.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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ORCID

Maya Bar ⓘ http://orcid.org/0000-0002-7823-9121
Adi Avni ⓘ http://orcid.org/0000-0003-2092-9768

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