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Common mechanisms activate plant guard receptors and TLR4

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Abstract

In metazoans, the innate immune system uses Pattern Recognition Receptors to detect conserved microbial products, whereas in plants Guard Receptors detect virulence factors or activities encoded by pathogens. In a recent study, Williams and colleagues report that plant Guard receptors can be activated by a mechanism remarkably similar to that of mammalian Toll-like Receptor 4.

The task of recognizing microbial pathogens is accomplished by the Pattern Recognition Receptors (PRRs) of the innate immune system [1]. While we consider innate immunity to be controlled by similar principles in all multicellular organisms, host defense in plants is often discussed in a context that differs from that in metazoans [2]. In the animal kingdom, innate immunity is most commonly considered in the context of Janeway's Pattern Recognition Hypothesis [3], which posits that one or more PRRs detect common microbial products such as bacterial lipopolysaccharides (LPS), lipoproteins, flagellin subunits and viral nucleic acids. This strategy of microbial detection does not distinguish virulent from avirulent microbial encounters, but rather considers all microbial encounters as potentially pathogenic. PRR activation leads to the upregulation of diverse antimicrobial activities designed to thwart a possible infection. While the strategy of Pattern Recognition is utilized by plants, innate immunity in this kingdom is often discussed in the context of the Guard Theory proposed by Dangl and Jones [4]. Unlike Pattern Recognition, the Guard Theory posits that innate immune receptors operate to either detect virulence factors encoded by pathogens, or the activities of such factors. Because plant innate immune receptors detect pathogen-encoded activities or factors, the Guard strategy has the ability to identify virulent microbes specifically, and mount appropriate responses to limit the infection. Thus, metazoan PRRs can be best-characterized as *microbe-detection receptors* whereas plants can encode *pathogen-detection receptors*.

Because of the conceptual distinction between the Pattern Recognition and Guard Strategies, the importance of discoveries in plants for understanding metazoan innate immunity (or vice versa) is sometimes overlooked. For example, based on the fundamental differences in the nature of the ligands detected by PRRs and Guard receptors, it is not entirely clear that the

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mechanisms of receptor activation would be related in any way. Interestingly, an exciting new study by Williams *et al* reveals that the mechanism by which a plant Guard receptor is activated is remarkably similar to the mechanism by which the mammalian LPS receptor Toll-like Receptor 4 (TLR4) is activated [5].

Williams *et al* studied the NOD-like Receptor (NLR) proteins RPS4 and RRS1 [5]. These NLRs are each required for immune responses to specific bacterial virulence factors called effectors, which are encoded by *Pseudomonas syringae* and *Ralstonia solanacearum* [6]. RPS4 and RRS1 each contain a Toll-IL1 Receptor-Resistance (TIR) domain. This domain is also found in the cytosolic tail of the transmembrane-domain containing Toll-like Receptors (TLRs) in metazoans. Microbial detection by an extracellular leucine-rich repeat domain in a TLR triggers the dimerization (or oligomerization) of the TIR domain, a process necessary for activating protective innate immune responses [1]. Through detailed structural and biochemical analysis *in vitro* and *in planta*, Williams *et al* made the intriguing finding that RPS4 and RRS1 interact with each other in resting (uninfected) plants [5]. This interaction was observed in the crystal structure of the complexed TIR domains of these NLRs, although other domains may also contribute to interactions between the full-length proteins. The existence of an RPS4-RRS1 dimer in resting cells suggests a role for this protein complex in the earliest stages of infection, at the level of pathogen detection. Indeed, RRS1 was found to bind to bacterial effectors, even in the absence of RPS4, suggesting a direct interaction between this NLR and microbial proteins. Whether RPS4 also binds to bacterial effectors is unclear. Interestingly, despite the ability of RRS1 to bind bacterial effectors, it was incapable of activating an immune cell death response called hypersensitivity when its TIR domain was overexpressed. In contrast, overexpression of the RPS4 TIR domain fully induced a cell death response. Taken together, these results suggest a model of activation whereby a stable RRS1-RPS4 heterodimer exists in plant cells. During bacterial infections, RRS1 binds with high affinity to specific bacterial effectors, an event that leads to the dimerization of the TIR of RPS4 to initiate innate immune signal transduction.

If one considers this proposed model in the context of what is known about TLR4 activation, a remarkably similar sequence of events emerges. Similar to the RRS1-RPS4 complex, TLR4 forms a complex in resting cells with a protein called MD-2, and genetic deficiencies of either MD-2 or TLR4 renders mammalian cells unable to respond to bacterial LPS [7]. Like RPS4, TLR4 is a signal transducer that activates innate immune responses through TIR-dependent activities [8]. However, TLR4 does not bind to its microbial activator – LPS – with high affinity [9]. It relies on its interaction with MD-2, which contains a high-affinity LPS-binding activity. Upon LPS binding, MD-2 crosslinks TLR4 to promote TIR-dependent innate immune signal transduction. Thus, MD-2 can be considered the conceptual analogue of RRS1 in that they both represent the ligand-binding subunit of a sensory-signaling heterodimer (Figure 1). TLR4 and RPS4 can also be grouped as the signaling subunits of this heterodimer.

These proposed functional similarities between the TLR4-MD-2 and the RRS1-RPS4 complexes are all the more striking when considering how different these components are in other regards. TLR4 is a membrane-bound receptor, whereas RPS4 is soluble. TLR4 functions to promote inflammatory cytokine expression, whereas RPS4 promotes cell death

responses. And perhaps most distinct, MD-2 recognizes a generic component of the bacterial outer membrane (LPS) whereas RRS1 detects virulence factors encoded by specific bacterial pathogens. This latter point highlights the difference between the Guard theory and the Pattern Recognition theory, with the RRS1-RPS4 complex representing a Guard receptor and TLR4-MD-2 representing the prototypical PRR. The fact that both RPS4 and TLR4 form stable complexes with high affinity ligand-binding proteins suggests that similarities do indeed exist between the innate immune receptors in plants and animals, even at the level we normally consider most distinct— microbial detection (Figure 1).

An interesting result of this new study is that it highlights how proteins that differ in their structure and subcellular sites of action can operate under similar molecular principles (Figure 1). This implication is strengthened by recent studies comparing the immune responses in mice and *Drosophila melanogaster*. Just as has been described for the RRS1-RPS4 and TLR4-MD-2 complexes above, the receptor-proximal sorting adaptor proteins that control Toll signaling in flies and mice are structurally distinct, yet have a similar mechanism of action [10].

A question that arises from this new study is why RRS1 and RPS4 did not evolve to be encoded by a single gene that encompasses both ligand-binding and signal-transducing activities. It is possible that the genetic separation of these activities allows for a modular system to develop, where multiple proteins that bind different bacterial effectors may utilize RPS4 to promote innate immune responses. The answer to this question may help explain why TLR4 and MD-2 are encoded by different genes. Thus, the long-term implication of the study by Williams *et al* may be that follow-up research will involve scientists who study both plants and animals, which should create a richer discussion of host-microbe interactions and immunity.

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Figure 1.

A common mechanism of plant NLR and mammalian TLR4 activation highlights similarities between the Guard and Pattern Recognition theories.

(A) Depicted are the of TLR4-MD-2 and RRS1-RPS4 complexes that exist in uninfected mammalian or plant cells, respectively. The proteins that comprise these complexes are unrelated and occupy distinct subcellular sites. (B) A common mechanism of activation occurs between the TLR4 and RPS4 systems, which highlights a common theme between classically defined Guard receptors and PRRs.