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Nuclear Actions in Innate Immune Signaling

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Innate immunity in plants and animals is mediated through pattern recognition receptors, which were thought to initiate signaling in the cytoplasm to activate defense pathways. Shen et al. (2006) and Burch-Smith et al. (2007) now provide compelling evidence that certain plant disease resistance proteins, which detect specific pathogenic effectors, act in the nucleus to trigger downstream signaling and defense pathways.

Distinguishing self from nonself is the fundamental principle of immunity. Both plants and animals rely on extracellular and intracellular pattern recognition receptors to sense invading microorganisms and trigger immediate innate immune signaling cascades. A common structural feature in numerous pattern recognition receptors is the leucine-rich repeat (LRR) implicated in signal perception. Unique to many intracellular pattern recognition receptors is the nucleotide-binding domain (NB, also called NBS, NBARC, NOD, NACHT-NAD) shared by plant disease resistance (R) proteins, mammalian NLR (NOD-like receptor or CATERPILLER) proteins, and animal proapoptotic proteins, such as mammalian Apaf-1 and *C. elegans* CED-4 (Chisholm et al., 2006; DeYoung and Innes, 2006; Jones and Dangl, 2006; Ting et al., 2006). Mammalian NLRs have been shown to sense conserved microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs)—such as flagellin, peptidoglycan-derived molecules, and bacterial RNA—and activate caspase1, RIP2 protein kinase, and NF-κB in the cytoplasm (Ting et al., 2006). In contrast, plant NB-LRR receptors directly or indirectly recognize specific pathogen effector proteins for effector-triggered immunity. Plant NB-LRR proteins are subgrouped into coiled-coil (CC)-NB-LRR and Toll-interleukin-1 receptor (TIR)-NB-LRR based on distinct N-terminal domains. How and where plant NB-LRR receptors respond to specific pathogenic effectors and initiate defense responses is of major interest (Chisholm et al., 2006; DeYoung and Innes, 2006; Jones and Dangl, 2006). Combining state-of-the-art in vivo imaging and mutational analysis, Shen et al. (2006) and Burch-Smith et al. (2007) report the surprising findings that CC-NB-LRR and TIR-NB-LRR receptors enter and act in the nucleus to trigger innate immune signaling. Both groups fused a nuclear export signal sequence to NB-LRR receptors, the barley MLA receptor and the tobacco N receptor, and showed that their residence in the nucleus is absolutely required for defense responses.

Shen et al. (2006) analyzed barley CC-NB-LRR receptors (MLA1, MLA6, and MLA10) that confer resistance to the powdery mildew fungus *B. graminis* f sp *hordei* (*Bgh*) expressing specific effectors (e.g., AVR_{A10} for MLA10). Using the conserved CC_{1-46} region (present in all known MLA receptors) as bait, the authors identified HvWRKY2, a WRKY transcription factor, in a yeast two-hybrid screen. Importantly, transient overexpression of HvWRKY2 and closely related HvWRKY1 compromised immunity mediated by multiple MLAs. The result suggests that HvWRKY1/2 are negative regulators of defense pathways shared by multiple CC-NB-LRR receptors. Given that only truncated MLAs interact with HvWRKY1/2 in the absence of specific effectors in vitro, the receptors may be kept in a quiescent state by intra- or intermolecular interactions or both in vivo. Using improved Förster resonance energy transfer measurement, Shen et al. (2006) went on to show that AVR_{A10}—which probably activates MLA10 by inducing a conformation change—triggers a specific association between MLA10 and HvWRKY2 in the nucleus in vivo. This association inactivates the HvWRKY1/2 repressors in the nucleus to promote effector-triggered immunity. Identification of the direct target genes of HvWRKY1/2 will help unravel the early transcriptional events initiated by CC-NB-LRR receptors. The activated CC-NB-LRR receptors likely coordinate both negative and positive regulators to fully trigger complex downstream events in the nucleus. Whether AVR_{A10} directly or indirectly interacts with MLA10 in the cytoplasm or nucleus for priming and for signaling remains to be determined.

Burch-Smith et al. (2007) shed exciting new insight into the interaction between the tobacco mosaic virus effector p50 and the tobacco TIR-NB-LRR N receptor. Using sensitive fluorescent protein tagging, both N and p50 proteins were visualized in the cytoplasm and the nucleus. Surprisingly, nuclear exclusion of the

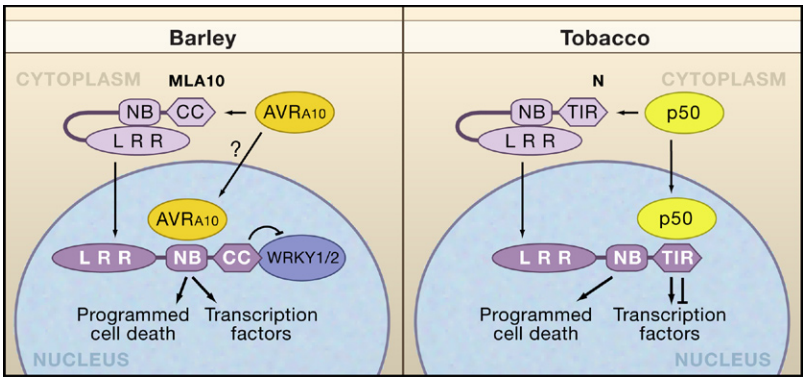

Figure 1. Nuclear NB-LRR Receptor Signaling in Effector-Triggered Immunity

The barley MLA10 (CC-NB-LRR) and tobacco N (TIR-NB-LRR) receptors are present in both the cytoplasm and the nucleus. Nuclear retention of either receptor is indispensable for downstream signaling and defense. Receptor activation by cognate AVR_{A10} and p50 effectors through intramolecular conformation changes, interactions with host proteins, and/or oligomerization could be initiated in the cytoplasm or in the nucleus. AVR_{A10} is required for nuclear MLA10 association with the transcription repressors HvWRKY1/2. NB-LRR receptors could interact with different host proteins for downstream signaling.

N receptor but not the viral effector p50 abolishes the effector-triggered immunity. Using Bimolecular Fluorescence Complementation imaging in planta and coimmunoprecipitation assays, Burch-Smith et al. convincingly showed that the TIR domain—previously proposed to be involved in signaling not ligand recognition—is essential and sufficient for association with p50. Host factors may assist the association in vivo. Based on several recent reports, a complex model for N receptor activation by the specific viral effector is emerging. The tobacco N receptor may first encounter the viral effector via the TIR domain. The viral p50 effector then disrupts the intramolecular TIR-NB and LRR association and interacts with the NB-LRR region (Ueda et al., 2006) to promote N protein oligomerization (Mestre and Baulcombe, 2006) and subsequently initiates downstream signaling in the nucleus. The precise subcellular locations of these consecutive events remain to be determined. It will be informative to examine the subcellular localization of the viral replicase carrying the p50 helicase effector domain to help define the initial contact compartment.

The provocative findings from these two studies prompt new questions about how conserved downstream responses—such as programmed cell death, MAP kinase, and genome-wide transcriptional activation—are initiated in the nucleus. Notably, a mammalian NLR, CIITA, acts in the nucleus as a master transcription activator that interacts with multiple DNA-binding transcription factors and histone-modification enzymes. CIITA was considered a unique and isolated case (Ting et al., 2006). Now the barley and tobacco studies suggest that nuclear sensing and signaling may be more common than previously anticipated.

Both *HvWRKY1* and *HvWRKY2* were found to be potently but transiently activated in barley by *Bgh* fungus early during infection and by flg22 (a conserved MAMP). Therefore, it was proposed that barley HvWRKY1/2 and the *Arabidopsis* homologs, AtWRKY18/40/60, are early MAMP-inducible transcription factors, which likely play a role in a negative feedback loop to limit or reset MAMP signaling. One function of the activated nuclear MLAs would be to counteract WRKY repression through direct protein-protein contact. Many *WRKY* genes are transiently induced by flg22 in *Arabidopsis*. Some of them may also play negative roles in plant defense and can be inactivated by the NB-LRR signaling pathways. Importantly, the unique expression pattern of *AtWRKY18* induced by a virulent bacterial pathogen *Pseudomonas syringae* and its overexpression studies indicated additional but positive functions for AtWRKY18 in plant defense (Xu et al., 2006). Using genomic and mutant analyses, Wang et al. (2006) demonstrated that AtWRKY18 is a positive regulator and a major direct target of the nuclear transcription cofactor NPR1 in systemic acquired resistance and salicylic acid signaling. Thus, both general and effector-specific responses may converge on the WRKY transcription factors.

The important findings reported by Shen et al. and Burch-Smith et al. have illustrated the complex multistep processes in the initiation of effector-triggered immunity with unexpected nuclear localization directly linked to DNA-binding transcription factors (Figure 1). However, WRKYs can act as activators or repressors. Their dynamic expression patterns, precise target genes, endogenous DNA-binding motifs, and roles in diverse microbe-host interactions are all crucial information to elucidate their distinct or overlapping functions. Considering temporal and spatial dynamics of multiple protein-protein interactions in different subcellular locales will help us to better understand the reality of intracellular NB-LRR sensing and signaling. Future research will explore new ligands or effectors for known and novel intracellular pattern recognition receptors. Integrated approaches including proteomics and structural information of NB-LRR complexes will unravel the dynamics and complexity of immune recognition and signaling processes, as well as subcellular and compartmentalized actions with diverse signaling partners.

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Magic Spots Cast a Spell on DNA Primase

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The bacterial signaling molecules ppGpp and pppGpp regulate transcription initiation in response to starvation by altering RNA polymerase activity. In this issue, Wang et al. (2007) show that (p)ppGpp also inhibits DNA replication elongation by interfering with DNA primase activity. Halting replication may help cells to maintain genomic integrity during periods of transient nutrient limitation.

Bacteria lead a feast or famine existence. Not surprisingly, they have elegant mechanisms to ensure survival in times of nutritional stress. For example, it has been known for half a century that starvation of various kinds triggers the "stringent response," which dramatically alters the genome-wide transcription profile (reviewed by Cashel et al., 1996). The stringent response has been best characterized in *Escherichia coli*, where amino acid starvation leads to changes in gene expression including inhibition of promoters for ribosomal and most transfer RNA (tRNA) operons and stimulation of promoters for many amino acid biosynthesis operons. The primary signaling molecules for these responses are the guanosine nucleotides pppGpp and ppGpp, which bind directly to RNA polymerase. These unusual nucleotides are collectively referred to as (p)ppGpp or "magic spots I and II" from their original identification on thin layer chromatograms by Mike Cashel in the late 1960s.

A variety of reports have hinted that (p)ppGpp's effects extend beyond transcription (see Wang et al., 2007 for references). For example, amino acid starvation has been reported to cause arrest of DNA duplication in *Bacillis subtilis* by stalling the replication machinery at positions on the chromosome called LSTer and RSTer (*l*eft and *r*ight *s*tringent *ter*minator) sites (Autret et al., 1999). To test whether nutritional stress terminates replication in a locus-specific manner, Wang and colleagues followed the progress of DNA replication in synchronized *B. subtilis* cultures using a time-resolved genomic microarray assay. The progress of the bidirectional replication forks could be determined with remarkable precision by visualizing the increase in DNA from one to two genome equivalents. Furthermore, the positions where replication forks halted in response to amino acid starvation could be easily identified in this assay. Contrary to the LSTer/RSTer site model, replication fork stalling was not determined by specified positions in the genome, but rather the position of fork stalling was determined by the interval of time that the forks were allowed to progress following initiation of replication. If the forks had already moved past the LSTer/RSTer sites when starvation was induced, they were still blocked from progressing further. Inhibition of replication in nonsynchronized cultures and the absence of a requirement for the replication termination protein Rtp were consistent with the interpretation that replication elongation was arrested not at a specific site but throughout the genome in response to nutrient stress.

How then does amino acid starvation lead to the termination of DNA replication? It has long been known