more about how HCMV regulates the cell cycle during infection. The study represents an excellent example of how using drugs in the laboratory can be beneficial, and it is not out of the question that its results might have implications for the use of such drugs in the clinic.

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Activation of Plant Nod-like Receptors: How Indirect Can It Be?

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Pioneering plant research has shown that many Nod-like receptors (NLRs) detect pathogens indirectly via recognizing modifications of other host proteins. In this issue, two groups show that the RPM1 NLR is activated by phosphorylation of the host protein RIN4, probably resulting from activation of a host kinase by pathogen effectors.

The innate immune systems of both plants and animals employ intracellular receptors of the NOD-like receptor (NLR) family to detect the presence of pathogens. NLRs are characterized by the presence of a central nucleotide binding and oligomerization domain (NOD, often referred to as a NACHT or NB-ARC domain) and C-terminal leucine-rich repeats (LRRs). The N-terminal domains are more variable, and differ between plant and animal NLRs. The human genome encodes 22 predicted functional NLR genes (http://www.genenames.org/genefamily/nlr.php), and mutations in several of these have been linked to multiple autoinflammatory and immunodeficiency diseases (Inohara et al., 2005; Schroder and Tschopp, 2010). Understanding how NLR proteins are activated is thus of intense interest to both plant and animal immunologists.

Work on plant NLR signaling pathways is contributing to our understanding of mammalian NLR signaling, particularly in understanding how NLR proteins "detect" pathogens. Several plant NLRs have been shown to detect pathogen proteins indirectly via sensing pathogen-induced modifications of other host proteins (DeYoung and Innes, 2006). This has raised the question of whether mammalian NLRs might also use such indirect recognition mechanisms. Recent work indicates that human NLRP3, which is activated by a diverse array of pathogen molecules, probably detects the presence of pathogens indirectly (Schroder and Tschopp, 2010). The common denominator among the diverse signals that activate NLRP3 appears to be excess production of reactive oxygen species (ROS) by mitochondria (Zhou et al., 2011). Elevated levels of ROS appear to be "sensed" by thioredoxin (TRX)-interacting protein (TXNIP), which binds to NLRP3 in a ROS-dependent manner (Zhou et al., 2010). Thus, in this example, pathogens and other cellular stresses are being sensed indirectly by detecting the presence of free TXNIP released from thioredoxin as a consequence of elevated ROS. Whether other mammalian NLRs use such indirect mechanisms of detection remain to be determined, but this seems likely given the diversity of activators for most characterized NLRs and the complete absence of evidence for direct binding of known activators to their corresponding NLRs.

Among plant NLRs, arguably the best characterized is the RPM1 protein of *Arabidopsis*. RPM1 is activated by at least two different effector proteins from *Pseudomonas syringae*, AvrB and AvrRpm1. Activation of RPM1 by either of these

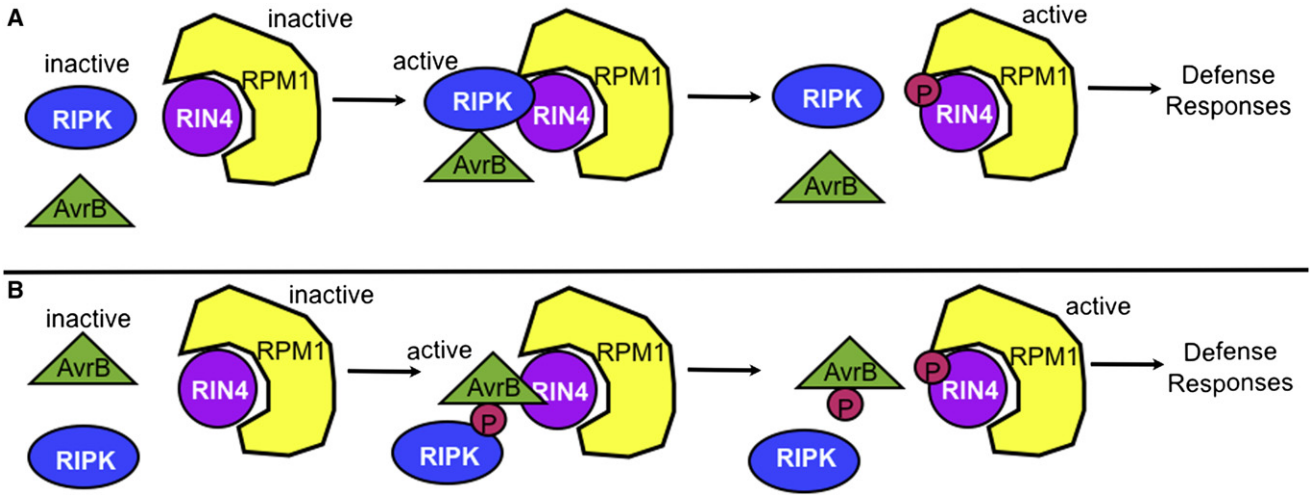

Figure 1. Two Models for Activation of *Arabidopsis* RPM1 by the *P. syringae* Effector AvrB
The work of Chung et al. and Liu et al. demonstrate that phosphorylation of RIN4 on threonine 166 activates RPM1 signaling, activating defense responses. In (A), preferred by Liu et al., RIN4 is phosphorylated by the host kinase RIPK, which is activated by AvrB. In (B), RIPK phosphorylates AvrB, which then phosphorylates RIN4.

bacterial proteins requires the presence of a second *Arabidopsis* protein, RIN4. It has been known for some time that AvrB and AvrRpm1 physically associate with RIN4 inside plant cells and that they induce phosphorylation of RIN4 (Mackey et al., 2002). However, it has remained unclear how these effectors cause phosphorylation of RIN4 and whether phosphorylation of RIN4 is what activates RPM1. Two papers in this issue by Chung et al. (2011) and Liu et al. (2011) now answer these long-standing questions. The answer to the first question appears to be "indirectly," while the answer to the second question is "yes."

Prior work had produced a crystal structure of AvrB bound to a small peptide from RIN4 (Desveaux et al., 2007). This structure revealed that AvrB contained an ATP-binding pocket and further suggested that a specific threonine residue on RIN4 was likely phosphorylated by AvrB if AvrB possessed kinase activity. Despite the best efforts of multiple labs, however, no one has been able to demonstrate kinase activity for AvrB in vitro. Chung et al. took the next logical step and modified the putatively phosphorylated threonine residue (T166), changing it to alanine to prevent phosphorylation, and to aspartate to mimic phosphorylation. Consistent with RIN4 T166 playing a central role in the activation of RPM1, the T166A substitution completely abrogated activation of RPM1 by AvrB. More importantly, the T166D substitution activated RPM1 in the absence of pathogen effectors, demonstrating that this minor modification of RIN4 was sufficient to trigger RPM1 signaling. Finally, using an antibody raised against a RIN4 peptide containing phosphorylated T166, Chung et al. were able to show that T166 does indeed become phosphorylated in the presence of both AvrB and AvrRpm1.

A perhaps surprising result in the Chung et al. work is that the T166A substitution has only a very minor impact on recognition of AvrRpm1 by RPM1. This observation indicates that while phosphorylation of T166 may be sufficient to activate RPM1, it is not the only way to do so. It also suggests that AvrRpm1 induces modification of other RIN4 residues in addition to T166. What these residues are remains to be determined.

The Liu et al. paper extends the findings of Chung et al. by identifying a host kinase, RPM1-induced protein kinase 1 (RIPK1) that phosphorylates RIN4 on T166 and is bound by AvrB. RIPK1 belongs to the receptor-like cytoplasmic kinase (RLCK) family, of which several have now been shown to play a role in plant innate immunity, including the *Arabidopsis* BIK1 and PBS1 proteins and the tomato Pto protein (DeYoung and Innes, 2006; Zhang et al., 2010). Significantly, all of these RLCKs are targeted by various *P. syringae* effectors, and PBS1 and Pto are both involved in activating NLRs (DeYoung and Innes, 2006; Zhang et al., 2010).

RIPK became of interest to Liu et al. when it was identified by mass spectrometry as part of a RIN4-containing protein complex, which they purified after activation by AvrRpm1. RIPK knockout mutants displayed enhanced resistance to virulent *P. syringae* strains, while overexpression lines displayed enhanced susceptibility, indicating that RIPK functions as a negative regulator of basal resistance pathways, as does RIN4. The physical association of RIN4 with RIPK1 led Liu et al. to test whether RIN4 was a substrate of RIPK in vitro, and they found that RIPK phosphorylated RIN4 at three positions, T21, S160, and T166. Similar to the approach taken by Chung et al., Liu et al. substituted a phosphomimetic residue (aspartate) at each of these positions and assessed whether the modified RIN4 protein could activate RPM1 in the absence of effectors. Consistent with the results of Chung et al., the triply substituted RIN4 activated RPM1.

Based on these data, Liu et al. hypothesized that AvrB might function by binding to RIPK and enhancing the kinase activity of RIPK and/or its specificity for RIN4, thus leading to enhanced phosphorylation of RIN4 (Figure 1A). The obvious question then becomes whether RIPK is required for AvrB-mediated phosphorylation of RIN4 and for activation of RPM1. The somewhat disappointing answer is

"only partially." The RIPK knockout mutant shows a reproducible reduction in RIN4 phosphorylation in response to AvrB, but no reduction in response to AvrRpm1, as assessed with an anti-phospho-T166 antibody. Consistent with this result, growth of a *P. syringae* strain expressing AvrB is only slightly enhanced in the *ripK* knockout, and the number of cells undergoing RPM1-induced cell death in response to this strain is slightly reduced. The authors speculate that the likely reason for these relatively modest effects is functional redundancy with other RLCK family members. Unfortunately, the large number of similar family members precludes an easy test of this hypothesis.

Another puzzling result is that AvrB and RIPK appear to compete for the same binding site on RIN4; when all three proteins are coexpressed, only AvrB immunoprecipitates with RIN4. It thus remains a possibility that AvrB phosphorylates RIN4 directly. Since the authors also show that RIPK can phosphorylate AvrB, one plausible model is that RIPK functions to phosphorylate AvrB, which then activates AvrB kinase activity (Figure 1B). The reduced phosphorylation of RIN4 in the *ripK* mutant background would then be the result of reduced AvrB activation.

Regardless of which model ultimately proves correct, the work of Chung et al. and Liu et al. have clearly established that NLR proteins can be activated by phosphorylation of other associated host proteins. It will be of interest to see how widespread this mechanism is among other plant and animal NLRs. At a more mechanistic level, the interesting question now becomes how phosphorylation of RIN4 alters its interaction with RPM1, and how this change leads to activation of RPM1.

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Evicting the Pneumococcus from Its Nasopharyngeal Lodgings

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Nasopharyngeal colonization by *Streptococcus pneumoniae* precedes invasive disease and mediates community transmission of the pathogen. In this issue, Moffitt et al. (2011) used proteomic analysis to identify conserved pneumococcal protein vaccine antigens that elicit T_H17-dependent responses capable of preventing such colonization.

Streptococcus pneumoniae (the pneumococcus) is one of the foremost bacterial pathogens in humans, causing massive global morbidity and more than a million deaths each year. However, it is essentially a commensal organism, asymptomatically colonizing the nasopharynx of a significant proportion of the human population, particularly young children and the elderly. Such carriage acts as a reservoir for transmission of the organism in the community, as well as a beachhead for subsequent penetration to otherwise sterile sites, including the middle ear cavity, lungs, blood, and brain, resulting in pneumococcal disease.

Development of effective and affordable pneumococcal vaccines has been a global health priority for many decades. Vaccines comprising purified capsular polysaccharides, the dominant pneumococcal surface antigens, were licensed in the late 1970s. However, these suffered from the shortcoming of poor immunogenicity in high-risk groups (particularly young children) because the component polysaccharides are T cell-independent antigens. Furthermore, the vaccine formulations (initially 14- and later 23-valent) provided incomplete coverage of the more than 90 known capsular serotypes of *S. pneumoniae*.