

R Protein Activation: Another Player Revealed

Jacqueline Monaghan^{1,2} and Xin Li^{1,2,*}

¹Michael Smith Laboratories

²Department of Botany

University of British Columbia, Vancouver, BC V6T 1Z4, Canada

*Correspondence: xinli@interchange.ubc.ca

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Resistance proteins play an integral role in plant innate immunity by perceiving pathogens and triggering defense responses. In this issue of *Cell Host & Microbe*, Kang et al. uncover CRT1, an ATPase essential for resistance to turnip crinkle virus in *Arabidopsis* mediated by the Resistance (R) protein HRT. CRT1 interacts with an array of R proteins in vivo, suggesting that it plays a role in R protein activation.

Plants do not possess specialized defense cells or an acquired immune system like that of animals. However, every plant cell is equipped with an arsenal of sensory molecules and receptors that can detect the presence of microbial pathogens and activate defense responses at multiple levels. One of the most effective defense responses is mediated by plant Resistance (R) proteins that recognize, either directly or indirectly, pathogen effector proteins generally thought to aid in virulence. Activation of R proteins triggers an array of physiological and biochemical changes that ultimately lead to a form of programmed cell death known as the hypersensitive response (HR) (Hammond-Kosack and Jones, 1996). HR lesions are localized at the site of infection, confining biotrophic pathogens to dead cells where they are deprived of essential nutrients—an event that in many cases is sufficient to stop an infection. The vast majority of cloned R genes are predicted to encode cytosolic NB-LRR proteins with structural and functional similarities to Nod receptors in mammalian innate immunity (Ausubel, 2005). NB-LRR proteins contain a central nucleotide-binding site (NB; also called a Nod domain), C-terminal leucine-rich repeats (LRR), and either a Toll/Interleukin-1-receptor-like region (TIR) or a coiled-coil domain (CC) at the N terminus (Jones and Dangl, 2006). The immediate response activated by NB-LRR receptors generally branches into two pathways depending on whether the protein is of the CC or TIR type, but these pathways later converge to activate a common set of defense genes.

Over the past 20 years, the use of *Arabidopsis* has prevailed as a model organism for plant molecular genetic analysis.

This is not unexpected given its small genome, short life cycle, simple transformation protocols, and the availability of genomic resources. Much of what we currently know about plant innate immunity was gained through the analysis of *Arabidopsis* mutants compromised in their ability to activate immunity responses. There are a number of *Arabidopsis*-microbe systems suitable for such analysis, covering a suite of viral, bacterial, fungal, or oomycete species against which certain *Arabidopsis* ecotypes are resistant. Classical mutant screens, in which mutagenized plant populations are examined for compromised resistance or enhanced susceptibility to pathogens, have revealed many key players in innate immunity; however, more sensitive and creative approaches are needed to identify subtler components of these pathways.

In *Arabidopsis*, resistance to turnip crinkle virus (TCV) is mediated in part by HRT, a CC-type NB-LRR that specifically detects the TCV coat protein (CP) and leads to the formation of HR lesions within 3 days of infection (Cooley et al., 2000). In the current issue of *Cell Host & Microbe*, Kang et al. (2008) exploit this interaction to identify components of the HRT activation pathway leading to HR. To do this, the authors used a strategy similar to that used by J. Dangl's group to identify AtRAR1 (Tornero et al., 2002) and HSP90 (Hubert et al., 2003), two components of a multiprotein complex that plays a key role in the R protein activation pathway. Kang et al. (2008) designed a mutant screen using a transgenic *Arabidopsis* line that expresses wild-type HRT and in which the expression of the TCV CP can be induced. Upon induction, recombinant

TCV CP expression is recognized in every cell by HRT, leading to systemic HR and plant death. The *crt1* mutant was identified based on its failure to mount an HR, reflected by its ability to survive the induction of CP expression (Kang et al., 2008). Positional cloning of *CRT1* revealed that it encodes an ATPase belonging to the same GHKL ATPase family as HSP90 (Kang et al., 2008). The authors demonstrate that CRT1 has enzymatic ATPase activity and is potentially localized to the Golgi (Kang and Klessig, personal communication; Kang et al., 2008), adding another regulatory player to the short list of defense-related proteins located in endomembranes and highlighting the relationship between subcellular components in the relay of R protein-mediated defense signals.

To gain further mechanistic insight into the role of CRT in innate immunity, Kang et al. (2008) investigated the genetic relationship between *crt1* and *ssi4*, a lesion mimic mutant in *Arabidopsis* that exhibits morphological defects and systemic HR lesions due to constitutive activation of the TIR-type R protein SSI4. Interestingly, *crt1* is able to suppress lesion formation and partially suppress stunted growth of *ssi4*, suggesting that CRT1 is required for both HRT- and SSI4-mediated resistance responses and thereby representing another point of convergence between the TIR- and CC-NB-LRR pathways. The authors also discovered that CRT1 interacts with HRT, SSI4, and two other R proteins, Rx and RPS2, in coimmunoprecipitation assays. Significantly, the interaction observed between CRT1 and HRT or SSI4 is abolished with a truncated recombinant protein similar to that expressed by *crt1*. It is therefore plausible that

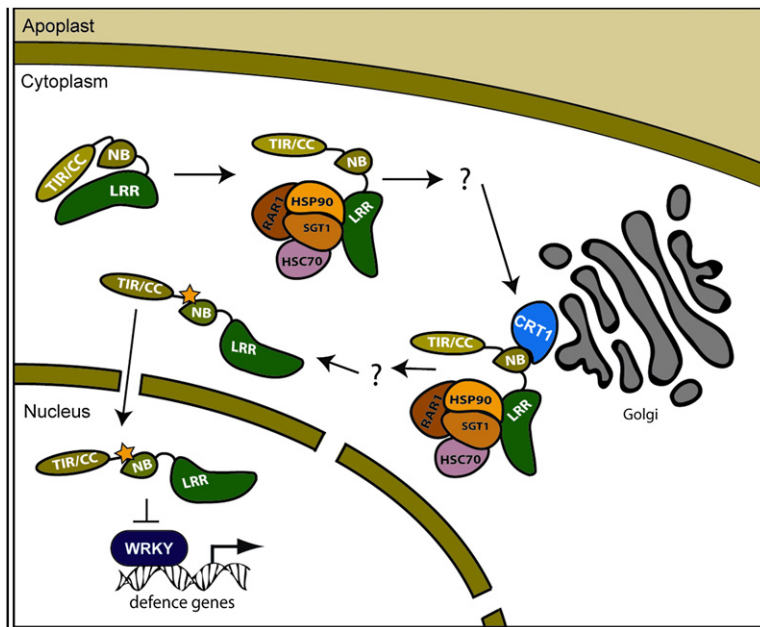


Figure 1. A hypothetical step-wise model of NB-LRR type R protein activation

NB-LRR is stabilized by the HSP90/SGT1/RAR1/HSC70 complex and modified or further stabilized by CRT1 prior to accumulation in the nucleus. Pathogen effectors could participate in this process to enhance (in the case of effectors recognized by plants) or suppress (in the case of effectors unrecognized by plants) host defense responses.

the reason why *crt1* plants fail to elicit a strong HR following either HRT or SSI4 elicitation is due to the disruption of these interactions. Taken together, these data point toward a novel role of CRT1 in NB-LRR-mediated resistance signaling in general.

R proteins are thought to exist in a repressed form in the absence of pathogens, either through inhibitory folding or interaction with negative regulators such as RIN4 (Marathe and Dinesh-Kumar, 2003). Although little is known about the activation of R proteins at the molecular level, the formation of stable R protein complexes, facilitated by a cytosolic protein complex involving HSP90, RAR1, Suppressor of G2 allele of SKP1 (SGT1), and heat shock cognate 70 kDa (HSC70), is required for the activation of a number of NB-LRRs (Shirasu and Schulze-Lefert,

2003; Noël et al., 2007). As CRT1 belongs to the same ATPase protein family as HSP90, it is possible that CRT1 might behave as another chaperone in R protein complexes. Alternatively, based on its residency in the Golgi, CRT1 could be involved in protein modification events to further stabilize or prime R proteins for activation. Interestingly, a number of exciting new studies have shown that some activated NB-LRR type R proteins, such as MLA10 in barley, N in tobacco, and RPS4 in *Arabidopsis*, accumulate in the nucleus where they most likely act to derepress defense gene expression (reviewed in Shen and Schulze-Lefert, 2007). What emerges is a step-wise model of NB-LRR R protein activation (Figure 1) in which chaperones and other R protein-binding partners play a key role. Following stabilization by HSP90 and other cocha-

perones, it is possible that R proteins are modified or further stabilized by CRT1. This process primes NB-LRR receptors for activation and accumulation in the nucleus where they can directly derepress inhibitory transcription factors (such as WRKYs) to activate defense gene expression (Shen and Schulze-Lefert, 2007).

This study introduces a novel component of R protein-mediated resistance and highlights the notion that R protein activation likely involves a number of finely controlled steps. Given the extreme fitness costs and autoimmune deficiencies associated with deregulated R proteins, it is not surprising that R protein activation would be under such tight regulatory control. Future work to unravel the functional significance of R protein binding partners such as CRT1 is therefore of particular interest.

REFERENCES

- Ausubel, F.M. (2005). *Nat. Immunol.* 6, 973–979.
- Cooley, M.B., Pathriana, S., Wu, H.-J., Kachroo, P., and Klessig, D.F. (2000). *Plant Cell* 12, 663–676.
- Hammond-Kosack, K.E., and Jones, J.D.G. (1996). *Plant Cell* 8, 1773–1791.
- Hubert, D.A., Tornero, P., Belkhadir, Y., Krishna, P., Takahashi, A., Shirasu, K., and Dangi, J.L. (2003). *EMBO J.* 22, 5679–5689.
- Jones, J.D.G., and Dangi, J.L. (2006). *Nature* 444, 323–329.
- Kang, H.-G., Kuhl, J.C., Kachroo, P., and Klessig, D.F. (2008). *Cell Host & Microbe* 3, this issue, 48–57.
- Marathe, R., and Dinesh-Kumar, S.P. (2003). *Mol. Cell* 11, 284–286.
- Noël, L.D., Cagna, G., Stuttmann, J., Wirthmüller, L., Betsuyaku, S., Witte, C.-P., Bhat, R., Pochon, N., Colby, T., and Parker, J.E. (2007). *Plant Cell*, in press. Published online on December 7, 2007. 10.1105/tpc.107.051896.
- Shen, Q.-H., and Schulze-Lefert, P. (2007). *EMBO J.* 26, 4293–4301.
- Shirasu, K., and Schulze-Lefert, P. (2003). *Trends Plant Sci.* 8, 252–258.
- Tornero, P., Merritt, P., Sadanandom, A., Shirasu, K., Innes, R.W., and Dangi, J.L. (2002). *Plant Cell* 14, 1005–1015.