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A Breakdown in Defense Signaling

One of the major mechanisms that plants use to escape disease involves a pathogen recognition system known as gene-for-gene resistance. This is controlled genetically by resistance (*R*) genes in plants, which encode proteins that recognize avirulence (Avr) products of an invading pathogen. Pathogen recognition triggers a wide range of both local and systemic responses whose cumulative effect is the inhibition of pathogen growth.

In the past decade, many *R* genes have been isolated that confer pathogen resistance to various plant species against a wide range of pathogens; however, the signaling processes that lead from initial pathogen recognition to the induction of the resistance response remain poorly understood (Glazebrook, 2001). Five exciting new articles address two downstream components of resistance signaling, RAR1 and SGT1 (Austin et al., 2002; Azevedo et al., 2002; Muskett et al., 2002, Tör et al., 2002; Tornero et al., 2002). Together, these articles provide critical evidence that protein degradation may be of pivotal importance for efficient pathogen responses and form the basis of a new paradigm for resistance signaling.

The majority of *R* genes identified encode intracellular proteins containing a predicted nucleotide binding site (NBS) followed by a series of leucine-rich repeats (LRR) at their C termini. In addition, NBS-LRR resistance proteins generally contain one of two types of N-terminal domains. These are either a domain that has homology with the Toll and Interleukin-1 Receptor proteins (TIR) or a predicted coiled-coil domain (CC). Functional studies of resistance proteins have indicated that the highly variable LRR domains determine recognition of the pathogen Avr product (Ellis et al., 1999; Jia et al., 2000; Dodds et al., 2001), whereas the more conserved TIR-NBS or CC-NBS regions are believed to propagate the perceived signal (Tao et al., 2000).

Two distinct resistance-signaling pathways have been described genetically, and at present, the *R* genes that are known to use each of these pathways correlate with the TIR and CC classes. The Arabidopsis genes *EDS1* and *PAD4* encode lipase-like proteins that interact with each other and mediate the downstream signaling of known TIR-type but not CC-type receptors (Feys et al., 2001). Conversely, the presumed membrane-bound protein encoded by *NDR1* is required for many CC-type resistance proteins but does not affect resistance triggered by known TIR-type resistance proteins (Century et al., 1997; Aarts et al., 1998). In addition, at least two CC-type resistance genes, *RPP8* and *RPP13*, function independently of *EDS1* and *NDR1*, suggesting the existence of at least one additional signaling pathway (McDowell et al., 2000; Bittner-Eddy and Beynon, 2001). At some point, these distinct pathways presumably converge because they give rise to similar resistance responses.

The simple picture of independent linear pathways has now been complicated by the introduction of *RAR1* and *SGT1*, which are required for signaling by sets of resistance genes that overlap the boundaries between the pathways defined previously (Table 1). The barley *RAR1* gene (*HvRAR1*) is required for powdery mildew resistance controlled by many but not all of the genes at the *Mla* resistance locus and at some other *R* loci in barley (Jørgensen, 1996). *HvRAR1* was isolated by map-based cloning and was found to encode a protein of unknown function that is conserved in all eukaryotes except yeast (Shirasu et al., 1999). Interestingly, initial clues regarding the function of the HvRAR1 protein came from the observation that animal RAR1 orthologs contain an additional domain with homology to the *Saccharomyces cerevisiae* protein SGT1 that is absent from plant RAR1 orthologs.

SGT1 was identified initially as a genetic suppressor of yeast *skp1* mutants (Kitagawa et al., 1999). The SKP1 protein is an evolutionarily conserved subunit of SCF-type E3 ubiquitin ligases that mediate the ubiquitylation of proteins that are targeted for degradation by the 26S proteasome (Deshaies, 1999; Bachmair et al., 2001). Biochemical analysis of yeast SGT1 indicated that it is a positive regulator of E3 ubiquitin ligase activity (Kitagawa et al., 1999). SGT1 homologs from Arabidopsis and barley have now been found to interact with RAR1 in the yeast two-hybrid system (Azevedo et al., 2002). In transiently transformed barley cells, double-stranded RNA inhibition of RAR1 or SGT1 resulted in the loss of *Mla6*-mediated resistance but did not affect the closely related *Mla1* resistance gene, consistent with previous analysis of *rar1* mutants (Azevedo et al., 2002). This suggested that SGT1 and RAR1 act together in mediating the *Mla6*-induced pathogen response.

Both SGT1 and RAR1 have been implicated more generally in defense signaling by the isolation of loss-of function mutants in Arabidopsis. These mutations have been isolated independently by several groups using genetic screens for loss of resistance mediated by different *R* genes (Austin et al., 2002; Muskett et al., 2002; Tör et al., 2002; Tornero et al., 2002). In contrast to barley, which contains only one *SGT1* gene, the Arabidopsis genome contains two *SGT1* orthologs, designated *SGT1a* and *SGT1b*, that seem to have

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Table 1. Summary of the Experimentally Proven Requirements for RAR1 and SGT1 for Specific *R* Gene–Mediated Pathways

R Gene[a]	SGT1b Dependency	RAR1 Dependency	EDS1 Dependency	NDR1 Dependency
RPP4 (TIR)	Yes	Yes	Yes	No
RPP5 (TIR)	Yes	Yes	Yes	No
RPP21	Yes	Yes	Unknown	Unknown
RPS4 (TIR)	No	Yes	Yes	No
RPS2 (CC)	No	Yes	No	Yes
RPS5 (CC)	No	Yes	No	Yes
RPM1 (CC)	No	Yes	No	Yes
RPP2 (TIR)	Yes	No	Yes	No
RPP6	Yes	No	Unknown	Unknown
RPP7	Yes	No	Unknown	Unknown
RPP28	Yes	No	Unknown	Unknown
RPP8 (CC)	No	No	No	No
RPP1A (TIR)	No	No	Yes	No
RPP10 (TIR)	Unknown	Unknown	Yes	No
RPP14 (TIR)	Unknown	Unknown	Yes	No
RPP13 (CC)	Unknown	Unknown	No	No

[a] TIR and CC denote the TIR-NBS and CC-NBS classes of resistance genes.

arisen through a recent gene duplication (Austin et al., 2002). Although mutations in *SGT1b* were identified in these loss-of-resistance screens, no mutations were found in *SGT1a* (Austin et al., 2002; Muskett et al., 2002). Interestingly, the spectra of *R* genes that require RAR1 or SGT1 do not coincide with those of genes that use EDS1/PAD4 or NDR1, nor do they match the TIR-NBS or CC-NBS classifications (Table 1). In fact, *R* genes from both classes and both pathways may require *RAR1* and/or *SGT1b* to varying degrees, so that some *R* genes require *RAR1* and *SGT1b*, some require *RAR1* or *SGT1b* only, and some require neither.

At this time, no known CC-NBS–type *R* gene has been shown to require *SGT1b* function. It could be hypothesized that the sample of tested *R* genes is too small to have led to the identification of an *SGT1b*-dependent CC-NBS–type *R* gene or that the yet uncharacterized *SGT1a* is specific for these *R* genes. Together, the genetic data suggest that RAR1- and/or SGT1b-mediated protein degradation may be involved in the responses that are controlled by some but not all *R* genes, that RAR1 function is dispensable for some SGT1b-mediated pathogen responses, and that SGT1b function is dispensable for some RAR1-mediated pathogen responses (Figure 1). It also needs to be noted that the requirement for RAR1 and SGT1b is not always absolute. The responses mediated by some *R* genes are affected only modestly by the *rar1* or *sgt1b* mutations, whereas others are inhibited completely.

Consistent with the two-hybrid interaction data, it was found that RAR1 and SGT1 form a complex in vivo (Azevedo et al., 2002). Additionally, SGT1 also is present as a subunit of SCF-type E3 ubiquitin ligase complexes, but these complexes apparently are distinct from the RAR1/SGT1 complex (Figure 1). Because SGT1 is an essential component of SCF-type E3 ubiquitin ligases, it can be assumed that the RAR1/SGT1b complex also functions in protein degradation; this notion is supported by the finding that RAR1 coimmunoprecipitates with components of another protein complex with a role in protein degradation, the COP9 signalosome (Azevedo et al., 2002). The COP9 signalosome is a multiprotein complex that has homology with the "lid" subcomplex of the 26S proteasome that was shown recently to interact with SCF-type E3 ubiquitin ligases in plants, animals, and the fission yeast *Schizosaccharomyces pombe* (Lyapina et al., 2001; Schwechheimer and Deng, 2001; Schwechheimer et al., 2001). Interestingly, the COP9 signalosome appears to be absent from the budding yeast *Saccharomyces cerevisiae* (Schwechheimer and Deng, 2001).

In plants, it has been possible to demonstrate that the COP9 signalosome is a positive regulator of SCF^{TIR1}, an E3 ubiquitin ligase that mediates responses to the phytohormone auxin (Schwechheimer et al., 2001; for review, see Kepinsky and Leyser, 2002). In addition, the COP9 signalosome also mediates a number of other physiological responses that are likely to be mediated by SCF-type E3 ubiquitin ligases (C. Schwechheimer, G. Serino, and X.-W. Deng, unpublished data). Furthermore, genetic evidence suggests that the COP9 signalosome is essential for the repression of photomorphogenesis in the dark, which is mediated by the putative non-SCF–type E3 ubiquitin ligase COP1 and an associated ubiquitin-conjugating enzyme variant, COP10 (Osterlund et al., 2000; Suzuki et al., 2002). Thus, the COP9 signalosome may be a central component of multiple processes that are mediated by E3 ubiquitin ligases belonging to different E3 families. Given this information, it is conceivable that RAR1 and SGT1b may participate in a novel E3 complex that also is regulated by the COP9 signalosome.

What could be the possible role of ubiquitylation or protein degradation in the context of pathogen response? At present, the molecular data are insufficient to draw strong conclusions regarding the precise role of RAR1 and SGT1 in defense signaling; however,

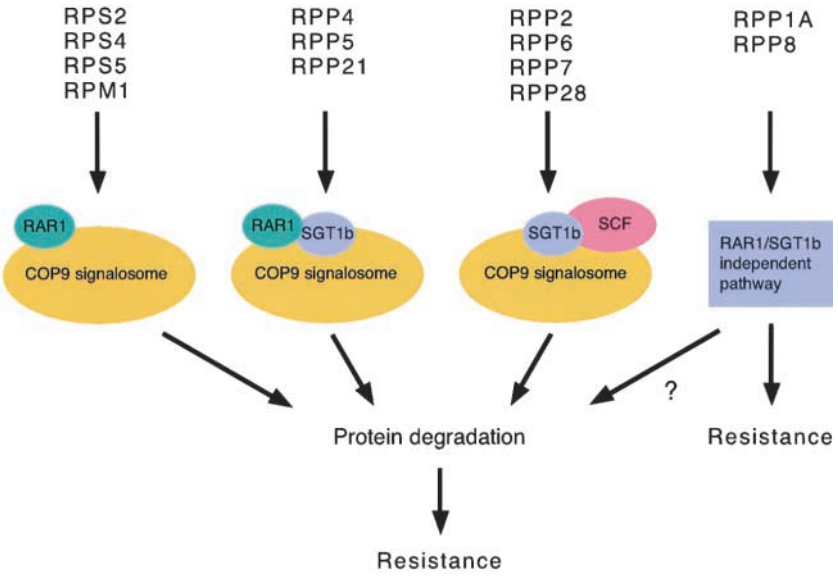

Figure 1. Differential Requirement of RAR1 and SGT1b as Downstream Components of Various R Protein Pathways.

The COP9 signalosome copurifies with RAR1 and SGT1b, but the anticipated requirement for this interaction in pathogen response needs to be verified experimentally.

there are several hypotheses that could be subjected to experimental tests. The most interesting clue for a role of protein degradation comes from the finding that the abundance of the R protein RPM1 declines rapidly after an AVR-induced resistance response (Boyes et al., 1998). However, Tornero et al. (2002) now report that RPM1 is undetectable in *rar1* mutants. This finding cannot be integrated easily into a model in which RAR1 mediates RPM1 protein degradation, and it suggests that RAR1 actually prevents RPM1 degradation in the absence of an AVR-induced stimulus, although this may be an indirect effect. Interestingly, RPM1 protein levels also decrease in the absence of the RIN4 protein, which appears to function with RPM1 in the recognition of its corresponding AVR products (Mackey et al., 2002). It will be important to discover whether other R proteins are affected similarly in *rar1* or *sgt1b* mutants.

Another role for RAR1/SGT1b-mediated protein degradation in resistance responses could be to modulate the abundance of positive or negative regulators of pathogen-induced gene expression. Expression of the *PR1* gene is constitutive in COP9 signalosome loss-of-function mutants, and this could be attributed to an inability to degrade a transcriptional activator that is degraded normally in the absence of a stimulus (Mayer et al., 1996). Interestingly, *PR1* also is expressed constitutively in *COP1* and *DET1* loss-of-function mutants (Mayer et al., 1996). Although the molecular role of DET1 is not understood at present, there is strong evidence that COP1 together with the E2 ubiquitin-conjugating enzyme COP10 functions as an E3 ubiquitin ligase (Osterlund et al., 2000; Suzuki et al., 2002). These findings suggest a potential role for COP1, DET1, and the COP9 signalosome in pathogen responses. It will be interesting to learn whether any of the related mutants are affected in *R* gene–mediated resistance.

The recent identification of SGT1b loss-of-function mutants also triggers a certain interest in the function of its Arabidopsis homolog SGT1a, for which mutants have not been identified. It will be interesting to determine if SGT1a and SGT1b mediate similar, dissimilar, or overlapping sets of pathogen responses. Given the anticipated wide spectrum of SCF-mediated processes in plants, it also will be interesting to learn about the role and the specificity of SGT1 proteins in other plant responses, such as cell cycle control and auxin response. At this time, no obvious phenotypes unrelated to disease resistance have been associated with the *rar1* or *sgt1b* mutations, but further analysis may reveal more subtle or conditional effects. Given the versatility and ease of manipulation of the resistance response as a model system, the function and specificity of other components of the degradation machinery certainly will be identified soon, along with their degradation substrates. An exciting time lies ahead as new directions open up for dissecting resistance-signaling processes in plants.

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