

Sensing Attack: The Role of Wall-Associated Kinases in Plant Pathogen Responses

The physical and chemical barrier provided by the cell wall is one of the first lines of defense against pathogen attack for plants. Plant cell walls provide physical protection against biotic and abiotic stresses but are also involved in a sophisticated sensing system for detecting both microbe-associated molecular patterns (MAMPs), such as flagellin (flg), and damage-associated molecular patterns (DAMPs), such as cell wall fragments, at the cell surface (Rui and Dinneny, 2020). This type of immune response is known as pattern recognition receptor-triggered immunity (PTI) and is distinct from nucleotide-binding, Leucine-rich-repeat triggered immunity (NTI), where pathogen-derived effectors are detected by receptors inside the cell. The organization of defense responses requires complex coordination between cell walls and the interior of the cell to activate downstream processes and so sensing and signaling between these two compartments is crucial. To date, few proteins that link these compartments have been identified. Understanding how signals are transduced between these compartments is of great interest to the study of pathogen resistance in plants and may reveal novel targets for crop breeding.

Plant cell wall-associated kinases (WAKs) are receptor-like kinases present in multiple plant species (Saintenac et al., 2018; Yang et al., 2019), possessing an extracellular domain and an intercellular domain that span the plasma membrane. WAK extracellular domains bind tightly to cell wall pectin (Decreux and Messiaen, 2005) and they are thought to transduce signals from the cell wall that, in part, regulate cell expansion (Wagner and Kohorn, 2001). More recently, it has been shown that WAKs have a higher affinity for small pectic oligosaccharides (such as those released during cell wall degradation) than for long-chain pectin

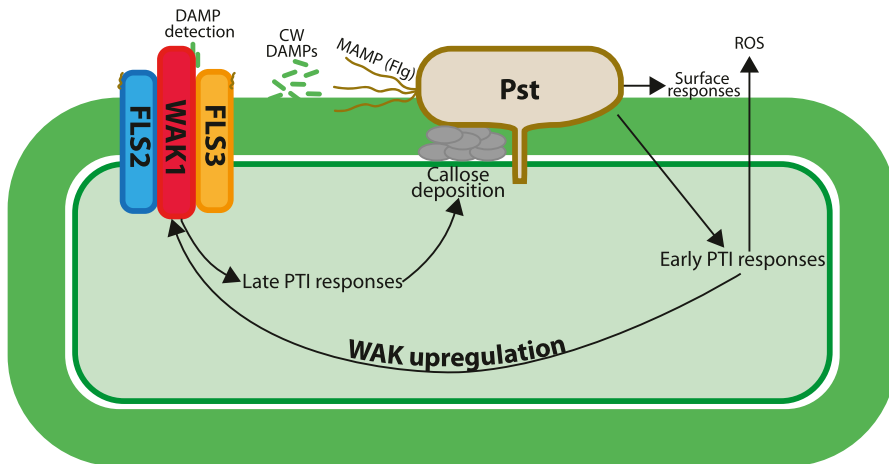
(Kohorn et al., 2014). This has led to the suggestion that WAKs are involved in sensing cell wall integrity (Rui and Dinneny, 2020), and several strands of evidence implicate them in pathogen responses (Rosli et al., 2013; Saintenac et al., 2018; Yang et al., 2019).

In this issue of *Plant Physiology*, Zhang et al. (2020) report an in-depth analysis of the role of tomato (*Solanum lycopersicum*) WAK1 in responses to *Pseudomonas syringae* pv. tomato (*Pst*) and identify putative interaction partners. Previous work from the group implicated WAK1 in pathogen responses (Rosli et al., 2013), and in this study the authors built on that using a CRISPR/Cas9 approach to generate homozygous knockouts of WAK1 in tomato for detailed characterization.

The authors first demonstrate that WAK1 is involved in PTI responses and not NTI by using two mutant strains of *Pst*. When *wak1* plants were challenged with a strain of *Pst* that is unable to activate NTI immunity, they showed enhanced disease symptoms compared to wild type, indicating that the mutant's PTI response was deficient. By contrast, when compared to wild-type *Pst*, no difference was seen using *Pst* lacking flg, a well-known MAMP, which therefore does not activate PTI. To confirm that WAK1 is involved in flg-mediated/PTI immune responses, plants were primed using synthetic flg and then challenged using *Pst* lacking flg. After two d, primed wild-type plants had significantly lower bacterial counts than unprimed plants, whereas priming had no effect in *wak1* mutants.

It appears that *wak1* mutants are not affected in their early PTI responses such as mitogen-activated protein kinase activation or the production of reactive oxygen species, but instead are strongly impaired in later responses. Specifically, the deposition of the cell-wall component callose was greatly reduced in *wak1* mutants. This suggests

Figure 1. Putative model of WAK1 function in pattern-recognition PTI to *Pst*. Early responses to *Pst*, such as the production of reactive oxygen species (ROS), are activated after the detection of the MAMP flg by the receptors FLS2 and FLS3. This leads to the upregulation of WAK1, which forms a complex with FLS2 and FLS3. WAK detects cell-wall (CW) DAMPs and activates late-stage PTI responses including callose deposition into the cell wall. Figure redrawn from figure 9 of Zhang et al. (2020).



that *WAK1* is functioning at the later stages of PTI immunity, which is consistent with previous suggestions that WAKs are involved in a cell wall integrity-sensing mechanism that monitors the status of the cell wall (Kohorn, 2015; Rui and Dinneny, 2020).

The authors also showed that flg treatment leads to an increase in *WAK1* expression, and that this is dependent on the pattern recognition receptors FLS2 and FLS3. In *fls2/3* knockout plants, disease symptoms occur more rapidly than in *wak1* plants, suggesting that FLS genes act upstream of *WAK1*. To decipher the biochemical relationship among FLS2, FLS3, and *WAK1*, the authors transiently expressed these proteins in *Nicotiana benthamiana* plants and carried out coimmunoprecipitation. An interaction was found between *WAK1* and both of the FLS proteins, indicating that they are within the same protein complex, but that their interaction is independent of the presence of flg.

A putative model for *WAK1* action is shown in Figure 1; it seems likely that expression of *WAK1* is upregulated during early-stage PTI responses and acts in a complex with FLS2 and FLS3 to mediate late-stage PTI responses such as callose deposition. It is not clear yet exactly which signals *WAK1* is detecting in tomato plants to stimulate callose deposition but, given our understanding of *WAK1* in other species, it is likely to be perceiving cell-wall-damage products such as pectin oligosaccharides that arise in the apoplast after pathogen attack (Kohorn et al., 2014).

Zhang et al. (2020) provide an in-depth analysis of the role of *WAK1* in tomato immunity to *Pst*. This study sheds some light on the workings of this fascinating but little understood group of receptor-like kinases that may provide a crucial signaling link between the cell wall and the cytosol. The economic impact of crop losses in tomato due to *Pst* infection can be substantial, and a better understanding of how resistance occurs could lead to novel breeding targets for developing pathogen-resistant cultivars.

This work raises additional questions. For example, the mechanism of *WAK1* transcriptional activation during late-stage PTI responses in tomato remains unknown. It is also not yet clear why *WAK1* acts later in the PTI response—is it because the protein levels are too low in these early stages, or could its activity require some signals generated in the early stage? For example, the movement of Ca^{2+} from the apoplast into the cytosol is an important early response in PTI (Ranf et al., 2011). This movement of Ca^{2+} leads to alkalization of

the cell wall, which alters the activity of pectin-remodeling enzymes (Hocq et al., 2017), possibly promoting the generation of the pectic oligosaccharides that are characteristic of the DAMP signals to which *WAK1* is known to bind. Piecing together the missing steps in this important defense response will be essential for informing future breeding strategies for developing pathogen-resistant crops.

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