IN BRIEF

**Phosphorylation and Nuclear Localization of NPR1 in Systemic Acquired Resistance**

When plants sense a pathogen attack, they activate defenses in the immediate area and in distal tissues; this systemic acquired resistance (SAR) requires salicylic acid (SA), which activates NONEXPRESSER OF PATHOGENESIS-RELATED GENES1 (NPR1) in Arabidopsis thaliana (reviewed in Fu and Dong, 2013). This activation involves the release of NPR1 monomers from oligomers, which is also regulated by the cell's redox state. Monomeric NPR1 is imported into the nucleus, interacts with bZIP transcription factors of the TGA family, and induces the expression of genes encoding antimicrobial pathogenesis-related proteins. Monomeric NPR1 also undergoes proteasome-mediated degradation, mediated by the NPR1 paralogs NPR3 (in the presence of SA) and NPR4 (in the absence of SA).

As if this were not complicated enough, measurements of SA levels during the establishment of SAR indicate the existence of other mechanisms regulating NPR1: During SAR, the biosynthesis of SA increases only a little in distal tissues, leading to the question of whether other factors activate NPR1 in those tissues. Shedding light on this, *Lee et al.* (2015) identify a role for SNF1-RELATED PROTEIN KINASE 2.8 (SnRK2.8), a serine/threonine kinase, in plant immunity. Following infection with *Pseudomonas syringae* pv *tomato* DC3000/avrRpt2, transcript levels of *SnRK2.8* rapidly increased in distal tissues, before the induction of *PR1*, but other SnRK2s were suppressed (see figure). Plants overexpressing *SnRK2.8* showed SA-dependent induction of *PR1* expression and increased disease resistance, but SnRK2.8 activation was independent of SA. Notably, *snrk2.8-1* mutants showed no effects on SA accumulation, but *PR1* expression and SAR were suppressed in the *snrk2.8-1* mutant.

Given the involvement of SnRK2.8 in immunity, the authors next examined the interaction between SnRK2.8 and NPR1. Indeed, coimmunoprecipitation and yeast two-hybrid assays showed that SnRK2.8 and NPR1 directly and strongly interact in yeast and in planta. Also, bimolecular fluorescence complementation assays showed that this interaction occurs in the nucleus and the cytoplasm. In vitro phosphorylation assays showed that SnRK2.8 phosphorylates NPR1. Moreover, two-dimensional gel electrophoresis of plants expressing a MYC-tagged version of NPR1 showed that induction of SAR also induces phosphorylation of NPR1 by SnRK2.8. Mass spectrometry and examination of mutant versions of NPR1 showed that SnRK2.8 phosphorylates NPR1 on Ser-589 and possibly on Thr-373; mutations of two other known sites of phosphorylation, Ser-11 and Ser-15, did not affect NPR1 phosphorylation by SnRK2.8.

What effect does phosphorylation by SnRK2.8 have on NPR1? The authors found that the wild type and the *snrk2.8-1* mutant showed similar patterns of NPR1-GFP localization. Moreover, NPR1 with substitutions at Ser-589 or Thr-373 failed to localize to the nucleus. The authors suggest that NPR1 localization occurs in two steps: SA-triggered monomerization and SnRK2.8-mediated phosphorylation. This mechanism allows the plant to activate SAR while avoiding the deleterious effects of high levels of SA in distal organs. This intriguing work sets the stage for future identification of the mechanisms activating SnRK2.8, possibly including nitric oxide, and examination of the role of other mobile immune signals in activating NPR1, the master regulator of SAR.

**REFERENCES**

