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EDITORIAL: REFLECTIONS ON *THE PLANT CELL* CLASSICS

Signal Transduction in Systemic Immunity

Studies of the plant immune system have provided profound insights into the mechanism of eukaryotic innate immune signalling, ranging from pathogen perception to activation of long-term protective responses. In the absence of memory cells as found in the adaptive immune system of animals, the demonstration that infection by certain pathogens and viruses triggered a long-lasting systemic immune response in plants, known as systemic acquired resistance (SAR), was particularly curious. Activation of SAR is associated with the expression of *pathogenesis-related* (*PR*) genes that encode for proteins with antimicrobial activity and provides long-term resistance against a broad spectrum of pathogens with predominantly biotrophic lifestyles. The early '90s saw the identification of salicylic acid (SA) as a key immune hormone in SAR necessary for the induction of *PR* genes. At the dawn of large-scale Arabidopsis mutagenesis, the stage was now set for mutant screens to identify genes involved in transduction of the SA signal.

A series of independent genetic screens were performed, the first of which appeared now exactly 25 years ago in *The Plant Cell* (Cao et al., 1994). The team of Xinnian Dong fused the *β-glucuronidase* (*GUS*) reporter to the SA-responsive *PR-2* promoter and screened for Arabidopsis mutants that lacked SA-induced expression of *proPR2-GUS*. This screen, along with several subsequent studies, repeatedly identified the *nonexpresser of PR genes 1* (*npr1*) mutant, indicating it played a central role in SA signalling. The identification and characterization of *npr1* alleles had a profound impact on decades of plant disease research. Indeed, *npr1* mutants are frequently used in genetic crosses to determine if SA signalling is involved in numerous biotic and abiotic stress responses in a variety of different plant species.

Three years after its identification, the *NPR1* gene was cloned and found to encode a protein with ankyrin repeats that mediate protein-protein interactions (Cao et al., 1997; Ryals et al., 1997). At the time these ankyrin repeats suggested that NPR1 bore remarkable resemblance to IκB in animals. IκB functions as an inhibitor of the transcriptional activator NF-κB, a master regulator of innate immune genes. In unchallenged cells IκB associates with NF-κB and sequesters it in the cytoplasm. However, upon microbial or viral infection IκB is targeted for degradation by the proteasome, releasing NF-κB to migrate into the nucleus where it activates immunity. While it was attractive to hypothesize that NPR1 might fulfil a similar inhibitory role as IκB, subsequent functional research revealed an unexpected level of complexity in the control of NPR1 activity in both gene activation and repression. These advanced functional studies were reported in a number of transformative publications, many of which appeared in *The Plant Cell*. At first it became clear that in addition to the ankyrin repeats, NPR1 protein also harbours a BTB domain for protein-protein interactions and a putative nuclear localization signal. The BTB domain enables NPR1 to form a high-molecular weight oligomer in the cytoplasm that is important for its homeostasis during infection (Mou et al., 2003). Accumulation of SA induces redox changes that trigger partial monomerization of NPR1, allowing NPR1 to migrate into the nucleus via its nuclear localization signal (Kinkema et al., 2000; Mou et al., 2003). In the nucleus NPR1 acts as a cofactor for TGA transcription factors that bind to specific DNA motifs in *PR* gene promoters. Not only was NPR1 shown to associate directly with TGA transcription factors, a series of papers demonstrated NPR1 facilitated their DNA binding and formed a transactivation complex responsible for *PR* gene activation (Després et al., 2000; Fan and Dong, 2002; Després et al., 2003; Boyle et al., 2009). Exciting recent findings indicate that the NPR1-TGA transactivation complex recruits chromatin modifiers to *PR* gene promoters (Jin et al., 2018), suggesting we have only uncovered the tip of the iceberg of NPR1-mediated transcriptional reprogramming. Furthermore, much remains to be discovered with regard to the control of NPR1 activity by numerous post-translational modifications, including oxidative modifications (*i.e.* disulfides and *S*-nitrosylation), phosphorylation, ubiquitination and sumoylation (Skelly et al., 2016).

While several labs focussed on the central role of NPR1 in SA-dependent SAR, the Van Loon and Pieterse team investigated its involvement in induced systemic resistance (ISR). ISR is activated by beneficial rhizobacteria that colonize the plant root and prime systemic aerial tissues to respond to pathogen infection with accelerated and enhanced defense responses compared to uncolonized plants. Unlike SAR, ISR does not require accumulation of SA, but rather requires responsiveness to the defense hormones jasmonic acid (JA) and ethylene. In a seminal report in *The Plant Cell*, NPR1 was surprisingly found to be necessary for activation of ISR against pathogenic *Pseudomonas syringae* (Pieterse et al., 1998). Evidence indicated that NPR1 functioned downstream of JA and ethylene to confer induced resistance. While the mechanistic role of NPR1 in the ISR signalling pathway still remains elusive and warrants further investigation, this report for the first time expanded NPR1's functions beyond a single immune signalling pathway. Recognition of NPR1 as a central hub for immune regulation was further cemented by the discovery that it regulates defense hormone cross talk. While SA provides resistance against biotrophic pathogens that feed on living host cells, JA activates defenses against insects and necrotrophic pathogens that kill the host cell before feeding. To prioritize activation of the appropriate defense pathway, the SA and JA signals are antagonistic. Again *The Plant Cell* reported that SA-

mediated inhibition of JA signalling was mediated by NPR1 (Spoel et al., 2003), further sparking interest in a new emerging area of research on immune hormone cross talk.

Because of its central role in diverse immune signalling pathways and its ability to trigger long-lasting immunity, NPR1 became an attractive biotechnological target for crop improvement. To date Arabidopsis *NPR1* has been expressed in numerous monocot and dicot food crops, ornamentals and even trees. In nearly all cases its expression triggered enhanced immunity against diverse pathogens but often also led to undesired autoimmune responses. Consequently, many NPR1 homologues have been identified in other plant species and their overexpression holds great promise. In addition, NPR1 is part of a larger family of NPR1-like proteins with similar protein domain structures. The functional characterization of these NPR1 paralogues is underway and already indicates that NPR1 does not act alone in transcriptional reprogramming during plant immunity. Twenty-five years ago Xinnian Dong's team would have been aware of the importance of identifying the *npr1* mutant, but they could not have foreseen it would spark decades of exciting research into the control of its function, activity, family members, and biotechnological utilization.

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