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exciting finding that calcium influx may activate a genetic program required for growth-factor-stimulated axon growth (Graef et al., 2003), it is tempting to suggest that homophilic N-CAM interactions might support axon growth through coordinating local Fyn-FAK activation with gene expression induced by FGFR activation and subsequent calcium influx. It will be of great importance to understand how the GDNF–N-CAM interaction activates both the local and the genetic programs required for axon growth.

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Closing Another Gap in the Plant SAR Puzzle

NPR1 is a key regulator of the salicylic acid (SA) dependent pathogen resistance pathway in plants. In this issue of *Cell*, Mou and Dong demonstrate that *Arabidopsis* NPR1 undergoes activation from an inactive oligomer to the active monomer as a result of cellular redox changes induced by SA during systemic acquired resistance.

Systemic acquired resistance (SAR) is a vital mechanism, which confers immunity throughout the plant toward a broad range of microorganisms following local infection by certain phytopathogens (Dong, 2001). The endogenous signal molecule salicylic acid (SA) has long been known to play a central role in plant defense with SA levels increasing in tissue upon pathogen infection. Genetic studies, mainly in the model plant *Arabidopsis thaliana*, have shown that SA is required for the induction of local defense responses, for activation of numerous defense-related genes including a set of pathogenesis-related (*PR*) genes, and in the establishment of SAR (Kunkel and Brooks, 2002). In addition, SAR can be induced in the absence of any pathogen by exogenous application of SA or its active analog 2,6-dichloroisonicotinic acid (INA).

Attempts by several laboratories to genetically dissect the SAR pathway downstream of the SA signal all resulted in the identification of numerous alleles of a single gene designated *NPR1*, *NIM1*, or *SAI1*. *NPR1* encodes a protein containing an ankyrin repeat domain and a BTB/POZ (*broad-complex*, *tramtrack*, and *bric-á-brac*/poxvirus, zinc finger) domain, both of which are involved in protein-protein interactions (Glazebrook, 2001). The importance of these domains for NPR1 function was solidified by the isolation of loss-of-function point mutations in highly conserved amino acids within these regions. *npr1* mutant plants fail to express several *PR* genes and display enhanced susceptibility to infection. They cannot be rescued by exogenous application of SA or INA consistent with an NPR1 function downstream of SA.

How does NPR1 exert its function and how does it transduce the SA signal? From the outset it was speculated that NPR1 could act as a transcription regulator to influence *PR* expression despite lacking any obvious DNA binding motif. Extensive work, including a series of elegant studies from X. Dong's laboratory, demonstrated that, in response to SA, NPR1 localizes to the nucleus via a functional nuclear localization signal (NLS), and that nuclear localization is a prerequisite for the activation of *PR-1* expression (Kinkema et al., 2000). Several yeast two-hybrid screens identified members of the TGA family of bZIP transcription factors as candidate interactors of NPR1. Indeed, NPR1/TGA2 interaction was subsequently directly visualized in plant protoplasts and also verified in planta, consistent with the fact that SA-dependent *PR-1* expression is positively influenced by the presence of an *as-1* element (a TGA factor binding site) within its promoter (Subramaniam et al., 2001; Fan and Dong, 2002).

Thus, pieces of the SAR puzzle are slowly beginning to fall into place. A major gap, however, concerns the mechanism by which SA accumulation directs NPR1 function within the SAR pathway. One should note that NPR1 protein is clearly present in uninduced plants and its concentration does not significantly increase upon SA or INA treatment. Furthermore, overexpression of *NPR1* alone does not activate *PR-1* expression nor induce resistance, clearly demonstrating the need for NPR1 activation by an unknown inducer (Cao et al., 1998). This suggests that SA somehow influences NPR1 function at the protein level. The article of Mou and Dong (2003) in this issue of *Cell* provides strong evidence that, upon SA/INA treatment or pathogen attack, alterations in the redox state of the cell may be the driving force leading to a transition from an inactive oligomer of NPR1 to an active momomeric form. Since the NPR1 protein contains 17 cysteine residues and a non-functional mutation (*npr1-2*) resulted in a cysteine to tyrosine conversion, the authors hypothesized that intra- or intermolecular disulfide bond formation could be important for NPR1 activity. Therefore, protein extractions in the presence/absence of the reducing agent dithiotreitol (DTT) were made from transgenic *npr1-1* mutant plants expressing a fully functional NPR1-GFP chimeric protein,

and subjected to immunoblot analysis using a GFP antibody. These studies revealed that in the absence of DTT, NPR1-GFP is only detectable in a high molecular weight complex. In contrast, extracts from plants pretreated with INA showed an additional cross-reacting band consistent in size to the monomeric form of NPR1-GFP. Addition of DTT to both extracts completely eliminated the complex resulting in the appearance of only the monomer form. It appears likely that NPR1 is maintained in a homooligomer complex via intermolecular disulfide bridges and that, upon stimulation, the reduced monomeric form is generated. The importance of certain cysteine residues within NPR1 was further evaluated by generating transgenic plants expressing NPR1 variants each having single amino acid substitutions at 10 different cysteine positions. Without SAR induction, constitutive levels of the monomeric forms were detected in two NPR1-GFP mutants (substitutions C82A and C216A). Interestingly, only in these two plant lines was *PR-1* expression found to be constitutive. Combined with results demonstrating that it is the monomeric form of NPR1 that translocates to the nucleus in an INA-dependent manner, one can conclude that this represents the active form of NPR1 which interacts with TGA factors leading to activation of downstream SAR-dependent target genes.

A common general plant response to pathogen attack is the rapid generation of active oxygen species such as H_2O_2, superoxide, and hydroxyl radicals and subsequent activation of counteracting antioxidant reactions. Both lead to disturbances in the redox state of the cell (Mittler, 2002). It is therefore not totally surprising that Mou and Dong observed a biphasic change in cellular reduction potential following INA treatment or pathogen challenge. Still, their demonstration that redox changes in the range measured in planta after SAR induction also lead to a reduction of the NPR1 oligomer complex in vitro is highly intriguing. How direct this effect is on NPR1 function remains to be determined. We still do not know how SA perturbs redox homeostasis and which reducing agents are actually involved.

Does the NPR1 oligomer actually exist at physiological concentrations in the cell? One caveat of the current study is that the existence of the complex was only demonstrated in an NPR1-GFP overexpressor line carrying the *npr1-1* mutation. Although non-functional with respect to SAR, the npr1-1 protein is additionally present and detectable within the NPR1-GFP oligomer. Thus, oligomerization may just be one cellular mechanism to inactivate excess NPR1.

One interesting point not raised by Mou and Dong concerns the recently demonstrated role of NPR1 as a cross-talk modulator between SA- and jasmonic acid(JA)-dependent defense pathways. SA has an antagonistic effect on JA-triggered signal transduction. Spoel et al. (2003) could show that NPR1 is required for this SA-mediated suppression of JA signaling, but that this function does not require nuclear localization of the protein. Can NPR1 undergo various conformational changes depending on the input signals and, if so, what is the mode of action of NPR1 in the cytosol?

As always, excellent papers are a source of stimulating thought and raise more questions than they actually solve. The paper of Mou and Dong is no exception, providing us with new insights on how NPR1 transduces the SA signal, thereby filling another gap in the SAR puzzles, but at the same time posing new challenges to be experimentally addressed.

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