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Stromules: Signal Conduits for Plant Immunity

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The chloroplast is a primary site for the production of immune signals in plants. In this issue of *Developmental Cell*, Caplan et al. (2015) report that chloroplasts send out stromules as signal conduits for transmission of these immune signals to the nucleus during effector-triggered immunity.

Plastids are a family of plant-specific heterogeneous double-membrane organelles that perform various vital functions. The best-known plastids are the chloroplasts, which carry out photosynthesis. A remarkable feature of plant plastids is their ability to form highly dynamic tubular extensions called stromules. The function of stromules is largely unresolved, although they are often associated with other organelles, raising the possibility that they may function as signal transmission channels to mediate inter-organellar communications (Hanson and Sattarzadeh, 2011). In this issue of *Developmental Cell*, the team led by Dinesh-Kumar reports a function for stromules during effector-triggered immunity (ETI) and programmed cell death (PCD) (Caplan et al., 2015). Their discovery advances our understanding of communications between chloroplasts and nuclei in the context of plant immunity.

Both plants and animals employ structurally and functionally similar nucleotide-binding and leucine rich-repeat (NLR) proteins to respond to perturbations caused by intracellularly delivered pathogen effectors. Upon activation, NLRs initiate ETI, which is often accompanied by PCD. An earlier study by Caplan et al. (2008) showed that the chloroplast protein N receptor-interacting protein 1 (NRIP1) is required for recognition of the tobacco mosaic virus (TMV) effector p50 by the NLR receptor N during ETI against this virus. Intriguingly, upon induction, NRIP1 is relocated from chloroplasts to the cytosol and the nucleus, where it forms a complex with p50. This complex activates N via interaction with its Toll-interleukin 1 receptor (TIR) domain (Caplan et al., 2008). In that study, the authors first observed N-triggered stromule formation.

To confirm this observation, Caplan et al., in the current work, used a quantitative method to determine whether stromule induction is specific to N activation as a way to coordinate the release of NRIP1 from the chloroplast. The results, however, suggest a different and more interesting conclusion. Caplan et al. found that stromules can be induced upon infection by the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 carrying different effectors, such as AvrRps4, AvrRpt2, or AvrRpm1. However, stromules are not induced by either a *Pst* DC3000 strain lacking these effectors or the type III secretion system mutant (*Pst* DC3000 *hrcC*), which is defective in delivering effectors. This suggests that stromules are generally induced during ETI, and this induction is not limited by the type of pathogen or the effector-NLR pair.

Further dissection revealed that stromule induction is not restricted to locally infected cells where ETI/PCD is activated, but also occurs in intact neighboring cells, suggesting that stromules may be induced by mobile signals produced during ETI/PCD. Activation of ETI results in production of an array of mobile immune signals, including defense-related hormones, secondary metabolites, and signaling proteins. Of those, ROS accumulation and production of the immune signal salicylic acid (SA) are of key importance in orchestrating cell death execution and defense response (Fu and Dong, 2013). Indeed, Caplan et al. found that exogenous application of either H_2O_2 or the SA analog 2,6-dichloro-isonicotinic acid (INA) can induce stromules in the absence of pathogen. These findings are in line with a prior observation from Itoh et al. (2010), who reported filamentous plastids in root cells treated with antimycin A, a mitochondrial respiratory inhibitor that induces production of ROS. Because chloroplasts themselves are the main source for persistent production of ROS and SA during pathogen infection, their response to ROS/SA therefore implies a feedback regulatory mechanism. Consistent with this idea, *chloroplast unusual positioning 1* (*chup1*), a mutant with enhanced stromule formation, confers enhanced PCD upon *Pst* DC3000/*AvrRpt2* infection. The authors thus propose that stromule induction and propagation of pro-defense signals may form a feedback amplification loop during immune induction.

Having provided compelling evidence that stromules are robustly induced by ETI/PCD-associated signals, the authors then show that many of the stromules establish intimate contact with nuclei during immunity. Close association and correlative dynamics with other organelles have been reported previously for stromules, and it was postulated that they serve as inter-organellar "highways" to facilitate transport of chloroplast-derived signals (Hanson and Sattarzadeh, 2011). Caplan et al. show that during ETI/PCD, enhanced stromule-nucleus association correlates with increased accumulation of NRIP1 in the nucleus. The authors further demonstrate that nuclear NRIP1 comes from the chloroplast by attaching a nuclear export signal (NES) peptide to the N terminus of NRIP1 in front of the transit peptide (TP) required for chloroplast targeting. Observation of NRIP1 in the nucleus implies that the NES has been cleaved off at the TP site when NES-TIP-NRIP1 passes through the chloroplast before relocating to the nucleus. Because the authors also find that nuclear ROS accumulation correlates with the ROS burst in the

surrounding chloroplasts, they hypothesize that a variety of chloroplast signals, including signaling proteins and small molecules, can be transported to the nucleus through stromules.

Finally, the authors explored how stromule induction is regulated. Because the actin cytoskeleton and the myosin XI motor are essential for stromule formation and movement (Kwok and Hanson, 2003; Natesan et al., 2009), CHUP1 was a good candidate. CHUP1 is a chloroplast outer envelope membrane protein possessing an actin-binding domain, which is required for chloroplast movement. However, as mentioned above, the *chup1* mutant exhibits constitutive rather than inhibited stromule formation. Although CHUP1 is not required for stromule induction, overexpressing CHUP1 TP blocks stromule induction. It has been reported that high levels of CHUP1-TP can saturate the chloroplast membrane insertion system and thus disrupt general protein insertion into the chloroplast envelope. Therefore, this result implies that unknown chloroplast envelope protein(s) may be required for stromule induction. Identification of this unknown chloroplast envelope protein(s) will be valuable in evaluating the specific contribution of stromules to ETI, as stromules can also form under a variety of developmental and environmental stimuli (Fester et al., 2007; Holzinger et al., 2007; Köhler and Hanson, 2000).

Caplan et al. (2015) provide intriguing evidence for a role for stromules in the execution of ETI/PCD. Figure 1 describes their working model. During the early stage of ETI, pro-defense signals (e.g., SA and ROS) promote proliferation of stromules, whose membrane extensions grow toward and become anchored close to the nucleus. Note that fusion between the stromule and the nuclear membrane has not been observed. Instead, formation of directional channels and subsequent release of stromal contents into the cytoplasm surrounding the nucleus may facilitate targeted delivery of chloroplast signals by reducing the diffusion distance to the nucleus and increasing the local concentration of chloroplast signals needed for activation of immune regulators in the nucleus. Moreover, a feedback amplification loop of pro-defense signal propagation and stromule proliferation may exist to contribute to the establishment of full immunity in both locally infected and intact neighboring cells.

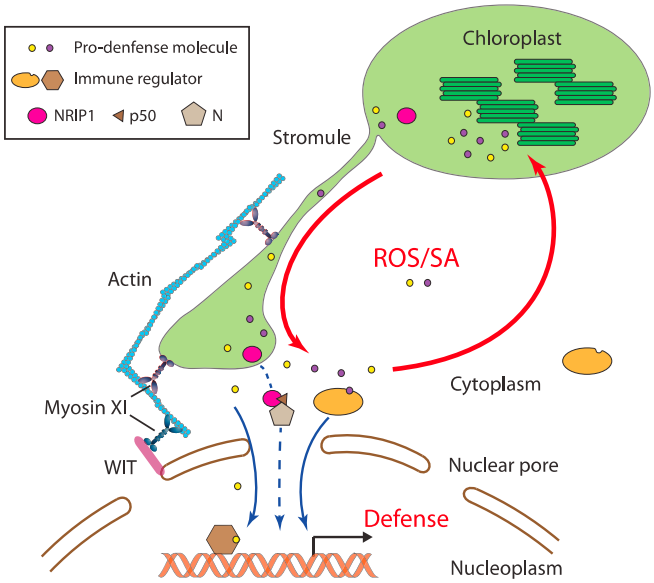

Figure 1. Stromules Facilitate Immune Signal Transmission to the Nucleus

Pro-defense signals (e.g., SA and ROS) generated in the chloroplasts during the early stage of effector-triggered immunity (ETI) promote stromule formation. Actin cytoskeleton, myosin XI, and myosin-interacting proteins (e.g., WIT) shape stromule membrane extensions and anchor stromule tips in close proximity to the nucleus in the absence of a membrane fusion. Immune signals, such as NRIP1, ROS, and SA, are released from stromules into the cytoplasm surrounding the nucleus to reduce signal diffusion distance and increase their local concentrations for activation of immune regulators in the nucleus. Stromule induction and propagation of pro-defense signals form a feedback amplification loop.

Like many other interesting discoveries, this work by Caplan et al. generates a number of new questions. First, at the molecular level, how are pro-defense signals perceived by chloroplasts, and how do they in turn activate membrane extension and stromule formation? Second, how is the directional extension of stromules to the nucleus achieved during defense? Third, what are the key components that regulate defense-specific stromule induction and nuclear association? Future studies should provide answers to these questions and further evaluate the biological significance of stromule-nucleus communication in plant immunity.

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