

Previews

I Will Survive: How NPR1 Condensation Promotes Plant Cell Survival

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The plant immune response regulator NPR1 resides in either the nucleus or in cytoplasmic puncta, depending on levels of the plant hormone salicylic acid. NPR1 nuclear roles include pathogenesis response (PR) gene regulation. In this issue of *Cell*, Zavaliev et al. determine that cytoplasmic NPR1-containing assemblies are consistent with multi-component protein condensates with roles to promote cell survival.

Plants do not have specialized immune cells; indeed, each cell is cemented in place by the cell wall. Therefore, each cell within the plant must be able to distinguish microbial friend from foe and respond accordingly. Part of this response can include programmed cell death to limit pathogen spread. Another part is signaling to other cells and even nearby plants to increase defense signals, a process called systemic acquired resistance (SAR).

NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1 (NPR1) is essential for systemic acquired resistance (reviewed in [Backer et al., 2019](#)). NPR1 contains an ankyrin repeat and a BTB/POZ domain, which are domains frequently found in E3 ubiquitin ligases. However, previous efforts to demonstrate E3 activity for NPR1 were unsatisfying. In addition, 20 years ago ([Kinkema et al., 2000](#)), NPR1 was reported to reside in either the cytoplasm or in the nucleus, depending on pathogen challenge and presence of the phytohormone salicylic acid (SA). Roles for NPR1 in these distinct locations present another unresolved mystery on NPR1. In this issue of *Cell*, Zavaliev et al. partially resolve these mysteries, demonstrating that NPR1 cytoplasmic puncta are multi-protein condensates and that NPR1 ubiquitin ligase activity requires its incorporation into these condensates. In the course of resolving some mysteries, they have raised new ones.

NPR1 assembles with other proteins into punctate structures in the cytoplasm

upon stimulation with threshold levels of SA. The authors call these structures SINC (SA-induced NPR1-condensates) ([Figure 1](#)). SINC are enriched with cell death regulators and stress response proteins. [Zavaliev et al. \(2020\)](#) reveal a surprising role for NPR1 in effector-triggered immunity by ubiquitylation of transcription factors involved in programmed cell death. The plant pathogen *Pseudomonas syringae* secretes effector proteins into a host's cell to attenuate host defense responses; however, if these effectors are recognized by the host, often a programmed cell death regime is initiated to limit pathogen spread (reviewed in [Cui et al., 2015](#)). Cells distal from the infection site increase SA production and express pathogenesis response genes in an NPR1-dependent manner. Indeed, Zavaliev et al. found that infection at these distal sites failed to trigger programmed cell death in an NPR1-dependent manner ([Figure 1](#)). Thus, NPR1 promotes cell survival and inhibits effector-triggered immunity in secondary infections. After the authors found several stress-related proteins in SINC, they examined NPR1 roles in additional stresses, including heat, oxidative, and DNA damage stress, finding that NPR1 promotes cell survival in response to a range of stresses. NPR1 was initially identified for affecting pathogenesis gene expression ([Cao et al., 1994](#)); these data suggest that NPR1 effects on other stress-induced genes should be examined in the future.

Zavaliev et al. hypothesize that SINC form via liquid-liquid phase separation,

but this is not formally demonstrated. They identify several disordered regions that may be redox responsive due to their content of cysteine residues, which they call RDRs for redox-sensitive disordered regions. When they tested the roles of these RDRs for mediating NPR1 assembly, substitution of the cysteine residues of RDR1 or RDR2 resulted in SINC assembly even without SA treatment. The RDR3 mutant, in contrast, behaved normally in the absence of SA but was not able to assemble SINC upon SA treatment. This demonstrates vastly different functions for the three RDRs; RDR1 and RDR2 are inhibitory to NPR1 condensate assembly, and RDR3 drives NPR1 condensate assembly.

Why these different regions have opposite effects on NPR1 assembly, despite some shared sequence properties, is an open question and will require further biophysical and structural characterization of these regions, including whether they are in fact disordered. The analyses and experiments in the manuscript imply the hypothesis that disordered regions are necessary for NPR1 condensation. Whether the SINC are formed via LLPS or another mechanism, there is little reason to believe *a priori* that IDRs are necessary for this process. While some IDRs are sufficient for mediating protein phase separation via homotypic interactions, and while disordered linkers influence the phase behavior of domain-motif systems (in which pairs of proteins with tandem repeats of modular binding domains and tandem repeats of short linear



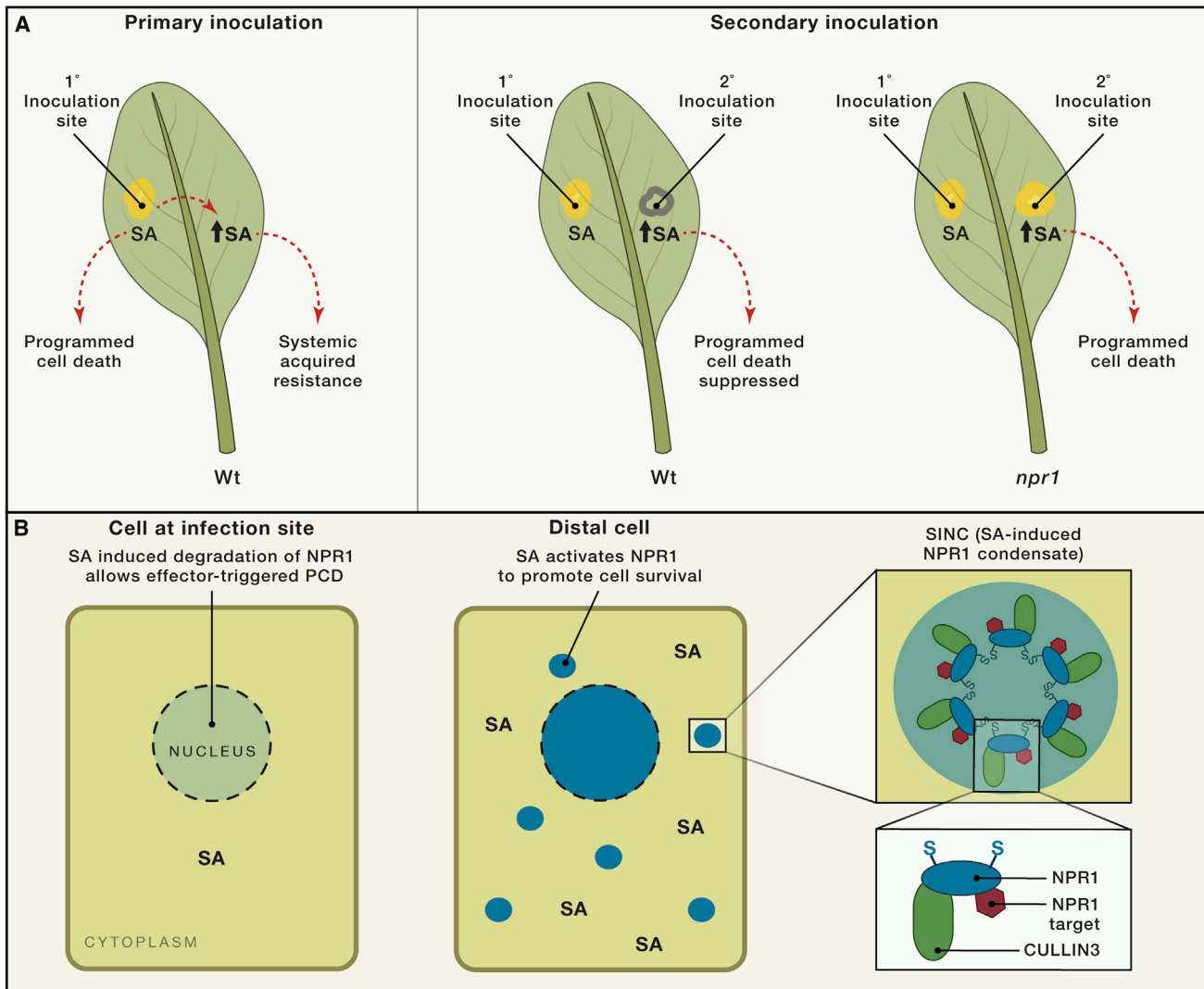


Figure 1. SA-Induced NPR1 Condensation Promotes Cell Survival

(A) NPR1 suppresses cell death at secondary infection sites. Pathogen infection leads to localized cell death at the infection site and salicylic acid (SA)-based promotion of systemic acquired resistance to future attacks at distal sites (left). During a secondary infection (right), wild type suppresses the programmed cell death regime and uses other mechanisms to limit pathogen spread; NPR1 is required for suppression of programmed cell death during secondary pathogen infection.

(B) During infection, NPR1 is primarily nuclear and promotes transcriptional regimes that lead to programmed cell death. At distal sites, SA signaling is dramatically increased and NPR1 localizes to cytoplasmic multi-component condensates, termed SINC (SA-induced NPR1 condensates). Within the SINC, NPR1 assembles into a CUL3-based E3 ubiquitin ligase complex to target stress-related proteins to the proteasome, resulting in increased cell survival.

motifs co-phase separate), phase separation can be mediated by folded domains (Riback et al., 2017), and IDRs can in fact be inhibitory to phase separation (Choi et al., 2020; Mittag and Parker, 2018). Hence, future work is required to understand whether SINC form through LLPS and what the underlying interactions are. Independent of such open questions, the insights into the SAR-related function of NPR1 in SINC solve several long-standing questions.

Why has it been difficult to show that NPR1 is a substrate adaptor of an E3 ligase when sequence analyses had long pointed in this direction? Zavaliev et al. show that the C-terminal domain of NPR1 inhibits CUL3 binding, likely via posttranslational modifications, but that NPR1 and CUL3 assemble into functional E3 complexes in the SINC. These are therefore biomolecular condensates that are active for ubiquitination as has been suggested also for condensates formed

by the CUL3 substrate adaptor SPOP in animals (Bouchard et al., 2018).

Understanding how SINC assembly is initiated will require additional mechanistic studies. The role of SA for inducing assembly and the requirement of redox-sensitive cysteine residues in NPR1 suggest that NPR1 may be a redox sensor that is not only used for reigning in effector-triggered immunity but that may also play roles in heat, redox, and DNA damage stress. The proteome of the

SINCs, which contain factors in these pathways, supports this idea.

Zavaliev et al. (2020) showcase a powerful combination of biochemistry, cell biology, and molecular genetics techniques to gain insight into NPR1 function in defense and in cell survival, revealing enzymatic functions limited to the condensed state. Future research will be necessary to understand the biophysical basis of condensate formation and its regulation by redox status as well as the precise roles played by NPR1 in promoting cell survival under various stresses.

DECLARATION OF INTERESTS

T.M. is a consultant for Faze Medicines.

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Stealth Killing by Uterine NK Cells for Tolerance and Tissue Homeostasis

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Human natural killer (NK) cells are critical for innate defense against pathogens through direct cytotoxicity of infected cells and are the predominant immune cell at the maternal-fetal interface. In this issue of *Cell*, Crespo et al. show that human NK cells in the decidual region of the uterus can clear a bacterial infection from the developing fetus by infusion of granulysin into placental trophoblast cells via nanotubes, thus removing the intracellular pathogen without damage to the placental cell. These findings reveal a mechanism for targeted immune protection of the developing fetus that maintains tolerance at the maternal-fetal interface.

Tissue-resident innate and adaptive immune cells populate diverse tissue sites, where they mediate immune protection and tissue homeostasis. The precise mechanisms by which specific types of resident immune cells mediate protection in different tissue environments remain open questions, including for tissues with continuous exposure to microbial species and immune-privileged sites for which microbial exposure cannot be tolerated. The pregnant uterus is one such immune-privileged environment, with the additional challenge of maintaining toler-

ance at the maternal-fetal interface, comprising the maternal decidua derived from the uterine mucosa, along with placental trophoblast cells from the developing fetus (Colucci, 2019) (Figure 1). The major immune cells in the early decidua are natural killer (NK) cells, a class of innate immune cells important for anti-pathogen immunity due to their ability to recognize and lyse infected cells. NK cells resident in the decidua (dNK) are required for establishment of the maternal-fetal interface (Hanna et al., 2006) as well as immune surveillance; however, the mech-

anisms by which dNK cells can protect while preserving tolerance have not been clear. New results by Lieberman and colleagues in this issue of *Cell* (Crespo et al., 2020) reveal unique features of both dNK cells and their interaction with trophoblasts that involve nanotube-mediated injection of granulysin (GNLY) for intracellular clearance of bacteria without damaging the trophoblast target cell, thereby preventing infection-induced pregnancy loss.

In humans, mature, cytolytic NK cells are prevalent in blood, while