PLANT IMMUNITY

A resistosome-activated 'death switch'

Pathogen perception triggers a monomeric nucleotide-binding leucine-rich plant immune receptor to form a pentameric wheel-like complex termed a resistosome, with the N-terminal α helices forming a funnel-shaped structure that may perturb plasma membrane integrity to cause hypersensitive cell death.

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Nucleotide-binding leucine-rich repeat (NLR) proteins are intracellular receptors that detect pathogen molecules and activate a robust immune response that includes the hypersensitive response (HR) cell death in plants[1]. Although plant NLRs are known to oligomerize through their N-terminal domains, such as the coiled coil (CC) domain[2], the molecular mechanisms that underpin plant NLR activation and the subsequent execution of HR cell death have remained largely unknown. In two recent remarkable papers published in *Science*, Wang et al.[3,4] have advanced our understanding of both the structural and biochemical bases of NLR activation in plants, creating a new conceptual framework. They reconstituted both the inactive and active complexes of the CC-NLR protein HOPZ-ACTIVATED RESISTANCE1 (ZAR1) with its partner receptor-like cytoplasmic kinases (RLCKs) and used cryo-electron microscopy to show that activated ZAR1 forms a structure called a resistosome — a wheel-like pentamer that can switch conformation to expose a funnel-shaped structure formed by the N-terminal α helices (α1) of the five CC domains. They propose a provocative model in which this funnel-like structure triggers HR cell death by translocating into the plasma membrane and perturbing membrane integrity, similar to pore-forming toxins.

Wang et al. first showed that ZAR1 is maintained as an inactive ADP-bound monomer in complex with the RLCK RESISTANCE RELATED KINASE 1 (RKS1), primarily through intramolecular interactions mediated by the leucine-rich repeat domain[3]. Uridylation of a second RLCK, PBS1-LIKE PROTEIN 2 ($PBL2^{UMP}$), by the bacterial pathogen effector AvrAC results in binding of $PBL2^{UMP}$ to RKS1 in the ZAR1–RKS1 complex. $PBL2^{UMP}$ binding induces conformational changes in the nucleotide binding domain of ZAR1, resulting in release of ADP and priming of the ZAR1–RKS1–$PBL2^{UMP}$ complex for activation. Subsequent binding of dATP/ATP to the primed ZAR1–RKS1–$PBL2^{UMP}$ complex induces pentamerization of the ZAR1–RKS1–$PBL2^{UMP}$ complex, leading to formation of the resistosome (Fig. 1). A conformational change — the 'death switch' — releases the α1 helices from being buried in the inactive ZAR1 complex to projecting out of the ZAR1 resistosome plane in a stunningly visual fold-switch. Structure–function analyses revealed that the α1 funnel is required for AvrAC-induced accumulation of ZAR1 complex in the plasma membrane and also for bacterial resistance.

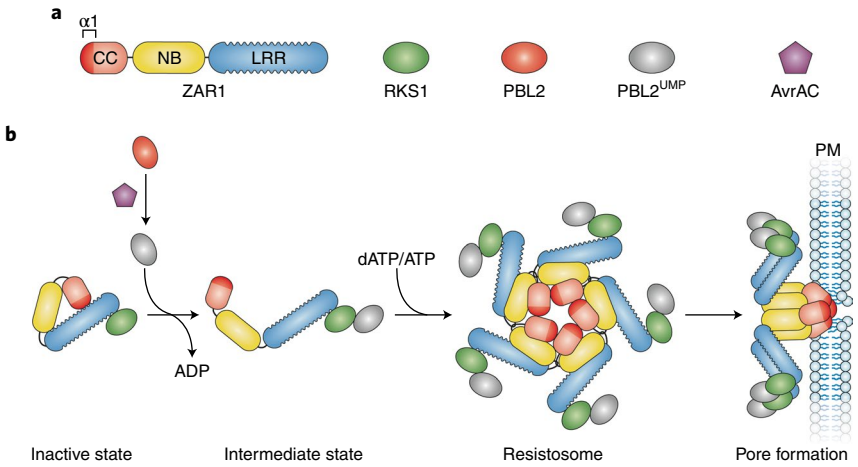

Fig. 1 | The *Arabidopsis* ZAR1 resistosome is proposed to directly trigger HR cell death through the formation of plasma membrane pores. a, Individual components involved in resistosome formation. **b**, Proposed formation and function of the resistosome. CC-NLR ZAR1 binds $PBL2^{UMP}$ uridylated by the *Xanthomonas campestris* effector AvrAC through RKS1 and undergoes conformational change, which results in ADP release from ZAR1 from the nucleotide-binding domain. This is proposed to shift NLR ZAR1 from an inactive to an intermediate state. dATP/ATP binding to intermediate ZAR1 complex results in pentamerization of ZAR1 into the resistosome wheel in which N-terminal α helices (α1, highlighted in red) become exposed to form a five-helix funnel-shaped structure ('death switch'). The ZAR1 resistosome translocates from the cytosol into the plasma membrane (PM) and the funnel is proposed to cause HR death of the plant cell by forming toxin-like membrane pores.

As noted by Wang et al.[4], the ZAR1 resistosome fold-switch is reminiscent of membrane pores and ion channels that are formed during pyroptosis and necroptosis cell death in animals. A similar mechanism also operates during NLR activation in fungi. Ligand-induced structural remodelling of the NLR-type heterokaryon incompatibility protein S (HET-S) of the fungus *Podospora anserina* releases a buried N-terminal transmembrane segment, which embeds HET-S in the plasma membrane and converts it into a pore-forming toxin[5,6]. The striking structural and mechanistic similarities between NLRs from plants, animals and fungi likely reflect a common evolutionary origin for these multi-domain ATPases, rather than independent convergence as previously proposed[7].

Whether inducible translocation of ZAR1 and other NLRs from the cytosol to the plasma membrane requires additional cellular machinery remains to be investigated. In addition, not all plant NLRs accumulate at the plasma membrane. Thus, it is possible that other membrane compartments are targeted by NLR

resistosomes — a hypothesis that deserves to be tested, especially when considering the massive perturbations of cellular processes that take place during pathogen infection[8].

How does the ZAR1 'death switch' model fit with the classical view of plant immune signalling? NLR-triggered immunity is associated with substantial downstream signalling, including activation of Ca^{2+} flux, mitogen-activated protein kinases, reactive oxygen species, phytohormone signalling and massive transcriptional reprogramming[9]. One possibility is that plasma membrane damage activates downstream signalling, which may function in cell-to-cell communication. Consistent with this idea, spatio-temporal analyses of phytohormone responses during NLR-triggered immunity indicate that these pathways are activated in distinct concentric areas surrounding the hypersensitive cell death[10]. Nonetheless, we cannot ignore the possibility that NLR resistosomes recruit downstream signalling components independently of any membrane damage activities.

ZAR1 is a singleton NLR that detects pathogen effectors indirectly[11]. However, other NLRs form receptor pairs and even networks in which the NLRs are functionally specialized as either sensors that detect pathogen effectors, or helpers that translate effector recognition into HR cell death and immunity[12]. It will be interesting to determine how the ZAR1 model may apply to these more complex NLR configurations, using this conceptual framework to ask new questions about NLR activation and cell death initiation. One system of particular interest is the complex genetic network formed by diverse sensor NLRs that rely on NLR-required for cell death (NRC) helpers to confer resistance to multiple pathogens and pests[13]. Many NRC-dependent NLRs—such as Mi, Rpi-blb2, Sw5b and Prf, carry large N-terminal domains prior to the CC, which would likely preclude activation through a ZAR1-type fold-switch. In addition, Sw5b truncations that lack the CC domain can still cause effector mediated cell death[14], and the CC domain of Rx, another NRC-dependent NLR, is dispensable for activation of HR cell death[4]. An interesting hypothesis is that the CC domains of these sensor NLRs have degenerated throughout evolution due to reliance on their NRC partners for signalling activity. Another possibility is that the sensor NLRs are recruited as one of the components of a hetero-oligomeric resistosome comprised of sensors and helper NLRs, as in the mammalian NAIP–NLRC4 inflammasome[15].

The elucidation of the ZAR1 resistosome structure and associated biochemical processes is a major advancement in our understanding of NLR biology. This knowledge should facilitate the development of improved synthetic disease resistance genes, for example by defining domain boundaries and guiding the design of chimeric NLRs. A thorough understanding of how these molecular machines function and how they are activated would ultimately enable remodelling the plant immune system to benefit agriculture. ❐

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Competing interests

The authors declare no competing interests.