Research Focus

Illuminating the molecular basis of gene-for-gene resistance; *Arabidopsis thaliana RRS1-R* and its interaction with *Ralstonia solanacearum popP2*

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Elucidation of the molecular basis of gene-for-gene interactions between disease-resistance (*R*) genes and pathogen avirulence (*avr*) genes has been a Holy Grail of plant pathology for the past decade. Recent studies of the *R–avr* interaction between *RRS1-R* and *popP2* by Laurent Deslandes *et al.* provide new insights and suggest a direct physical association of the encoded proteins in support of a simplistic receptor–ligand model. However, careful consideration of the experimental findings reveals that they could also be explained by molecular linker proteins that mediate formation of a PopP2 and RRS1-R uniting complex.

Half a century ago, Harold Flor's historical landmark publication revealed that the interaction between flax and flax rust is governed by single resistance (*R*) genes in the plant and complementary avirulence (*avr*) genes in the pathogen [1]. Based on this strictly genetic model of gene-for-gene resistance, many scientists envisaged R proteins as receptors that specifically bind to a matching Avr ligand to activate the defense machinery of the plant [2]. Isolation of more than 40 *R* genes [3] has revealed that most encode proteins with a predicted nucleotide-binding (NB) site and leucine-rich-repeats (LRRs). LRRs from other proteins have been found to participate in protein–protein interactions [4] and, thus, R protein structure is in agreement with the postulated receptor–ligand model. Yet, until recently, a direct interaction between an NB–LRR-type R protein and a corresponding Avr effector had only been shown for the rice Pi-ta protein and the matching AVR-Pita protein from the fungal pathogen *Magnaporthe grisea* [5]. Further empirical evidence for direct interaction of NB–LRR-class R proteins and corresponding Avr proteins is scarce, and this stimulated formulation of the guard model, in which NB–LRR proteins act as guardians of host proteins, which themselves are targeted by pathogen Avr proteins (Box 1). Currently, molecular evidence in favor of the receptor–ligand or the guard hypothesis is limited to a few model systems and therefore which model represents the more common mode of R protein action remains uncertain.

Structural features of PopP2, the RRS1-R Avr component, imply interference with the SUMOylation status of the proteome of the host

Recent findings about the *Arabidopsis thaliana* NB–LRR-type R protein RRS1-R and its cognate *Ralstonia solanacearum* Avr protein PopP2 provide fresh insights into the molecular principles that govern Avr–R interactions. Like the majority of *Arabidopsis* NB–LRR proteins, RRS1-R contains an N-terminal TIR (similarity to Toll interleukin-1-receptor) domain. Yet, in addition to the TIR–NB–LRR region, RRS1-R also contains a WRKY transcription-factor-like region at its C-terminus, a feature unique among known R proteins. Laurent Deslandes and colleagues [6] identified the RRS1-R Avr determinant PopP2 by systematic knockout of *R. solanacearum* candidate type-III effectors [6]. PopP2 belongs to the YopJ/AvrRxv family, a type-III effector class that is conserved between mammalian and plant pathogens and that structurally resembles the yeast ubiquitin-like protease 1 (Ulp1) [7]. Ulp1 mediates proteolytic maturation or removal of a small ubiquitin-related modifier (SUMO), an ubiquitin-like tag that is attached to target proteins post-translationally [8]. Various types of stresses induce accumulation of SUMO conjugates in *Arabidopsis* [9] and humans [10,11], suggesting that SUMO conjugation is a phylogenetically conserved stress response. Given that PopP2 belongs to a type-III effector family that is conserved between plant and animal pathogens, it is tempting to speculate that Ulp1-like bacterial type-III effectors act antagonistically to SUMO modification systems of the host to silence the intruder alert system.

In planta localization studies and Y2H data indicate spatial interplay between RRS1-R and PopP2

Many molecular studies of bacterial Avr proteins have uncovered eukaryotic targeting signals that mediate trafficking to subcellular destinations within the host cell [12]. PopP2 contains a putative N-terminal nuclear localization signal (NLS), a motif that binds to importin-α to facilitate movement into the nucleus [13]. Tagging of PopP2 with the fluorescent tracers GFP (green fluorescent protein) and RFP (red fluorescent protein) facilitated subcellular localization *in planta* and confirmed that PopP2 is targeted to the nucleus of the host. In contrast to PopP2, RFP- or GFP-tagged variants of the matching RRS1-R protein were not detectable by fluorescence microscopy, indicating that RRS1-R is either a low-abundance protein

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Box 1. History of the guard model

R genes were originally defined as single loci that confer resistance to otherwise susceptible plant genotypes [18]. This definition does not imply any information about the structure or the function of *R* gene-encoded proteins. Isolation of more than 40 *R* genes revealed that the vast majority encodes nucleotide binding–leucine-rich repeat (NB–LRR) proteins [3]. Because of their prevalence and their structure it was postulated that NB–LRR-type R proteins determine recognition of pathogen-derived Avr ligands by direct interaction [19].

However, the first Avr–R interaction was reported between *Pseudomonas syringae* AvrPto and the matching tomato Pto kinase, a structurally atypical R protein [20,21]. Notably, Pto-mediated defense depends on the NB–LRR protein Prf [22], which inspired the formulation of the guard hypothesis [23]. In this model, Pto represents a component of basal plant defense, which is inactivated upon binding to AvrPto, an effector protein that is injected into the plant cell by the bacterial type-III secretion system (Figure I). Thus, the postulated primary function of AvrPto is in virulence rather than avirulence, a hypothesis that has been confirmed for AvrPto and many other bacterial avirulence proteins [24]. The NB–LRR protein Prf is presumed to monitor ('guard') the virulence target Pto and to activate the defense system upon detection of an AvrPto–Pto complex. Recently, many variations of the guard model have been proposed, all involving the R protein detecting Avr protein activity rather than structure [24–26]. However, although conceptually fascinating, the role of Prf as a receptor for Pto or an AvrPto–Pto complex remains speculative, which has cast doubt on the applicability of the guard model in the context of Pto-mediated resistance [27]. However, corroborative evidence for the guard concept has emerged from analysis of the *Arabidopsis thaliana* RIN4 protein, which interacts with both the *Arabidopsis* NB–LRR-type R protein RPM1 and the cognate *P. syringae* AvrRpm1 and AvrB proteins [28]. Remarkably, RIN4 also associates with *Arabidopsis* RPS2, an NB–LRR-class R protein that mediates perception of the *P. syringae* AvrRpt2 effector [29,30]. Together these findings indicate that the activity of the *P. syringae* effector suite and the *Arabidopsis* R protein repertoire converges on a few pivotal host pathogenicity targets (reviewed in Ref. [31]).

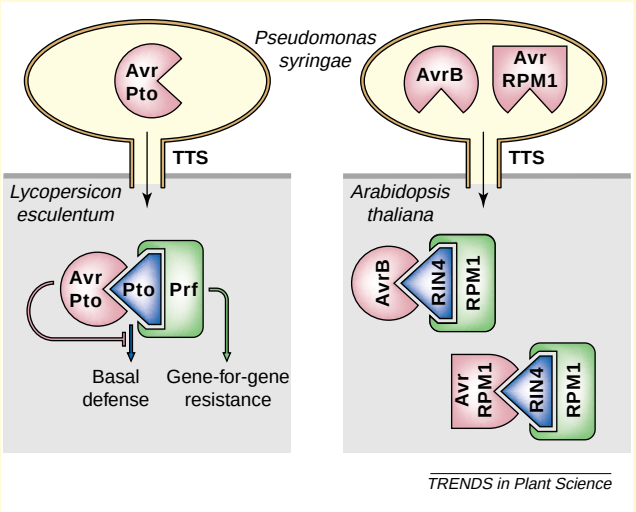

Figure I. The guard model in the conceptual framework of Pto- and RPM1-mediated resistance. The *Pseudomonas syringae* type-III secretion system (TTS) injects different effector proteins (pink) into the plant cytoplasm. Once inside the host, these effectors interact with a host target protein (green), thereby suppressing the basal plant defense. The role of the NB–LRR protein (blue) in this model is to 'guard' the Avr target protein. Abbreviations: LRR, leucine-rich repeat domain; NB, nucleotide-binding site.

or that the conformation of the fusion protein prevents chromophore visualization. Remarkably, RRS1-R–RFP was traceable to the nucleus when coexpressed with a nuclear-targeted PopP2–GFP, indicating that PopP2 either induces RRS1-R accumulation or modifies its conformation so that the fluorescent tag becomes visible. By contrast, Deslandes *et al*. detected RRS1-R–RFP in the cytoplasm when coexpressed with a GFP-labeled NLS-deletion derivative of PopP2 (PopP2 ΔNLS), which is also cytoplasmically localized [6].

Thus, *in planta* expression studies indicate that PopP2 has two distinct effects on RRS1-R. First, it facilitates, by an as yet unknown mechanism, visualization of an RRS1-R–GFP fusion protein. Second, the nuclear-targeted PopP2 and its cytoplasmically localized NLS deletion derivative seem to guide co-expressed RRS1-R proteins to the same cellular compartments, respectively. The spatial interdependency of PopP2 and RRS1-R points to a direct interaction between the proteins (Figure 1a). Indeed, Y2H studies suggest that RRS1-R interacts physically with PopP2 [6]. Thus, the molecular principles of the RRS1-R–PopP2 interplay seem generally to resemble the interaction of the fungal AVR-Pita protein and its matching rice NB–LRR-type R protein Pi-ta [5].

Interpreting the PopP2–RRS1-R interaction in the framework of the guard model

As outlined above, the findings about RRS1-R and PopP2 point to a direct Avr–R interaction in support of the receptor–ligand model. However, do these experimental findings justify rejection of the guard model for the RRS1-R–PopP2 interaction? Y2H studies seem to provide the most compelling evidence for a direct interaction between RRS1-R and PopP2. However, spatial proximity (not necessarily direct interaction) of bait and prey hybrid proteins is sufficient to activate Y2H-based reporter systems [14]. Thus, as emphasized by Deslandes and colleagues [6], the RRS1-R and PopP2-dependent Y2H reporter activation might be because of a conserved eukaryotic protein that facilitates formation of an RRS1-R and PopP2-containing complex *in planta* and in yeast rather than a direct interaction between RRS1-R and PopP2.

Are there obvious candidates for a molecular linker between RRS1-R and PopP2 that are conserved between *Arabidopsis* and yeast? PopP2 contains an NLS domain and thus is likely to associate *in planta* with importin α, which itself might be guarded by RRS1-R (Figure 1b). Importin α and its yeast homolog, karyopherin are 56% identical and thus karyopherin could potentially substitute for importin α in a postulated function as an adaptor between RRS1-R and PopP2. In support of this model, the PopP2 NLS deletion derivative PopP2 ΔNLS, does not trigger the RRS1-R-mediated defense reaction (L. Deslandes, S. Genin and Y. Marco, pers. commun.). However, *in planta* expression of PopP2 ΔNLS facilitates visualization of a GFP-tagged RRS1-R protein that, when expressed on its own is not traceable. Thus a PopP2 NLS-deletion

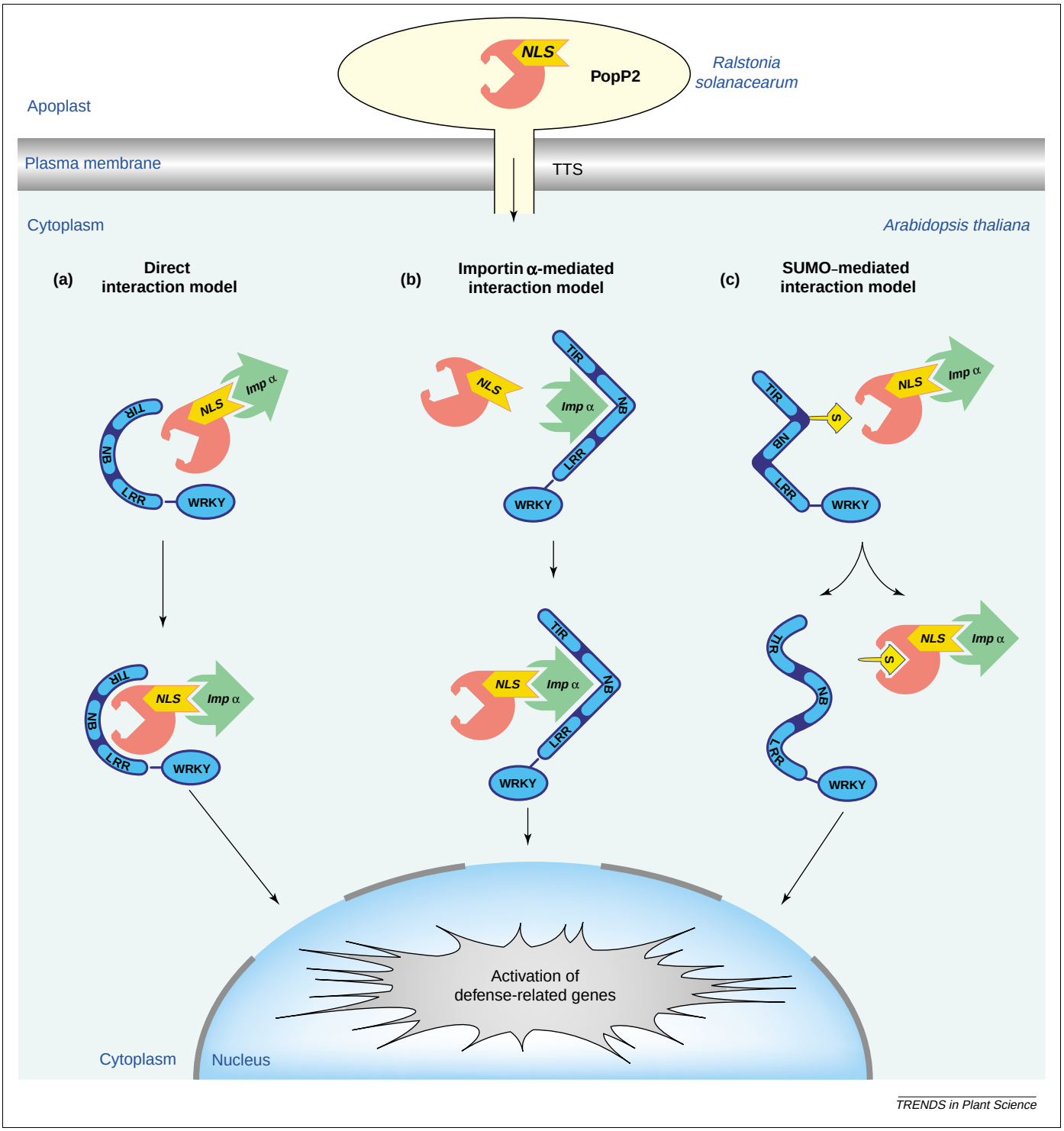

Figure 1. Proposed biochemical models of the RRS1-R–PopP2 interaction. RRS1-R might perceive PopP2 by direct interaction **(a)**. Alternatively, the interaction between RRS1-R and PopP2 is mediated by SUMO **(b)** or importin α **(c)**. Direct or indirect interaction of RRS1-R and PopP2 leads to nuclear import of RRS1-R and activation of defense-related genes. Abbreviations: Impα, importin α; LRR, leucine-rich repeat domain; NB, nucleotide-binding site; NLS, nuclear localization signal; S, SUMO; TIR, homologous to Toll/interleukin-1-receptor; TTS, type-III secretion system; WRKY, WRKY DNA-binding domain.

derivative, which is probably incapable of binding importin α, has undoubtedly an effect (direct or indirect) on RRS1-R. Hence, our model in which importin α is simply an NLS-dependent clamp between RRS1-R and PopP2 requires refinement to match this experimental finding.

Another protein that might bridge PopP2 and RRS1-R can be deduced from homologies between PopP2 and the *Xanthomonas* type-III effector XopD [15]. A recent study on XopD has shown that this bacterial effector deSUMOylates host proteins [16]. Thus, PopP2 might also act as a SUMO isopeptidase that marks SUMO as a potential virulence target of PopP2. In many cases, SUMO conjugation has been shown to repress transcription factor activity [17]. Thus, given that RRS1-R contains a transcription factor-like WRKY domain it is tempting to speculate that the activation of RRS1-R is triggered by

PopP2-mediated deSUMOylation (Figure 1c). In accordance with this idea, RRS1-R contains potential SUMO target sites. *Arabidopsis* and yeast SUMO proteins share significant sequence homology [9]. Thus, yeast SUMO moieties that are potentially conjugated to RRS1-R might serve as a PopP2 interaction site that mediates activation of the Y2H reporter genes. However, there is a caveat to this model. A PopP2 NLS deletion derivative does not trigger RRS1-R governed resistance, although our model implies that nuclear targeting of PopP2 is irrelevant to its avirulence activity.

Taken together, the correct interpretation of the available experimental data on the *RRS1-R–popP2* interaction poses an intellectual challenge. Importin α and SUMO are potential PopP2 virulence targets that might mediate physical association with RRS1-R *in planta* and in yeast. Alternatively RRS1-R and PopP2 might interact directly. In our view, data for this gene-for-gene interaction are presently consistent with either the receptor–ligand or the guard model.

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