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# Plant disease resistance protein signaling: NBS–LRR proteins and their partners

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Most plant disease resistance (R) proteins contain a series of leucine-rich repeats (LRRs), a nucleotide-binding site (NBS), and a putative amino-terminal signaling domain. They are termed NBS-LRR proteins. The LRRs of a wide variety of proteins from many organisms serve as protein interaction platforms, and as regulatory modules of protein activation. Genetically, the LRRs of plant R proteins are determinants of response specificity, and their action can lead to plant cell death in the form of the familiar hypersensitive response (HR). A total of 149 R genes are potentially expressed in the *Arabidopsis* genome, and plant cells must deal with the difficult task of assembling many of the proteins encoded by these genes into functional signaling complexes. Eukaryotic cells utilize several strategies to deal with this problem. First, proteins are spatially restricted to their sub-cellular site of function, thus improving the probability that they will interact with their proper partners. Second, these interactions are architecturally organized to avoid inappropriate signaling events and to maintain the fidelity and efficiency of the response when it is initiated. Recent results provide new insights into how the signaling potential of R proteins might be created, managed and held in check until specific stimulation following infection. Nevertheless, the roles of the R protein partners in these regulatory events that have been defined to date are unclear.

## Addresses

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## Abbreviations

<b>Apaf-1</b>	Apoptotic protease activating factor-1
<b>avr</b>	avirulence gene
<b>AvrB</b>	Avirulence protein B from <i>P. syringae</i> pv. <i>glycinea</i>
<b>AvrPphB</b>	Avirulence protein B from <i>P. syringae</i> pv. <i>phaseolicola</i>
<b>AvrRpt2</b>	Avirulence protein 2 from <i>P. syringae</i> pv. <i>tomato</i>
<b>BON1</b>	BON-1 ASSOCIATED PROTEIN1
<b>BOP1</b>	<i>BONZAI1</i>
<b>CC</b>	coiled-coil
<b>HSP90</b>	Heat Shock Protein90

<b>LRR</b>	leucine-rich repeat
<b>NBS</b>	nucleotide-binding site
<b>Nod</b>	nucleotide-binding oligomerization domain
<b>npr1-1</b>	<i>non-expresser of PR-genes1-1</i>
<b>PBS1</b>	AvrPphB SUSCEPTIBLE1
<b>R</b>	resistance gene
<b>RAR1</b>	REQUIRED FOR MLA12 RESISTANCE1
<b>RIN4</b>	RPM1-INTERACTING PROTEIN4
<b>RPM1</b>	RESISTANCE TO <i>P. syringae</i> EXPRESSING AVRRPM1
<b>RPS2</b>	RESISTANCE TO <i>P. syringae</i> EXPRESSING AVRRPT2
<b>Rx</b>	RESISTANCE TO POTATO VIRUS X
<b>SGT1b</b>	suppressor of G2 allele of <i>suppressor of kinetochore protein1 (skp1)</i>
<b>SNC1</b>	SUPPRESSOR OF <i>npr1-1</i> CONSTITUTIVE1
<b>TIR</b>	<i>Drosophila</i> Toll and mammalian Interleukin 1 receptor

## Introduction

The disease resistance genes (*R*) are the specificity determinants of the plant immune response. This simple but sophisticated immune system involves an allele-specific genetic interaction between a host *R* gene and a pathogen avirulence gene (*avr*) [1]. When this genetic interaction takes place, a defense response is triggered. This response is characterized by rapid calcium and ion fluxes, an extracellular oxidative burst, transcriptional reprogramming within and around the infection sites and, in most cases, a localized programmed cell death, which is termed the hypersensitive response (HR) [2,3]. It is thought that the sum of these events leads to a halt in pathogen growth. In the absence of specific recognition, a basal defense response also occurs, which is apparently driven by pathogen-associated molecular patterns (PAMPS) such as flagellin and lipopolysaccharides (LPS) [4]. The basal defense response overlaps significantly with R-protein-mediated defense, but is temporally slower and of lower amplitude. Basal defense does not prohibit pathogen colonization but does limit the extent of its spread [5]. Thus, R-protein action apparently accelerates and amplifies innate basal defense responses.

The simplest mechanistic explanation of the genetic interaction between *R* and *avr* genes is that the latter encode or generate ‘specific ligands’ that interact physically with a ‘receptor’ that is encoded by the corresponding plant *R* gene. Experimental data that support this model are rare [6,7], however. In fact, many avirulence proteins are actually required for maximal virulence on susceptible hosts that lack the corresponding *R* gene [8]; hence, they are actually virulence factors that contribute to disease. One plausible generality is that R proteins have evolved to recognize the functions of pathogen virulence factors. If this is true, then R proteins appear not to have evolved to recognize Avr proteins directly, as predicted by

receptor–ligand models, but rather to recognize the action of virulence factors as they modify or perturb host cellular targets. This model of indirect recognition has been termed ‘the guard hypothesis’, as it hypothesizes that R proteins have a surveillance role in cellular homeostasis [9,10].

The guard model suggests the following plausible cellular scenarios. First, R proteins are likely to be part of a multi-protein complex that should include proteins that are targeted by pathogen virulence factors. Second, the Avr proteins, presumably acting as virulence factors, specifically target one or more host proteins. These targets are probable partners of R proteins. Third, the perturbation of these cellular targets of pathogen virulence factors may or may not be required for virulence. Fourth, in either case, target perturbation leads to R-protein activation. Fifth, R proteins either constitutively bind to their partner(s) and then dissociate after modification of the complex by the type-III effector or form a new interaction with a cellular target that leads to activation.

In this review, we focus on the role of protein partners in nucleotide-binding site (NBS)–leucine-rich repeat (LRR) regulation in both the presence or the absence of an appropriate pathogen-derived stimulus. We also consider how the inappropriate activation of R proteins might be regulated. We suggest that the molecular mechanisms that govern NBS–LRR function might follow a ‘soft-wired signaling complex’ model [11], in which signaling molecules translocate into a receptor-containing complex and undergo reversible binding interactions and modifications. This dynamic view of signal transduction diverges from the historic ‘hard-wired signaling concept’, in which receptor–protein complexes were thought to be

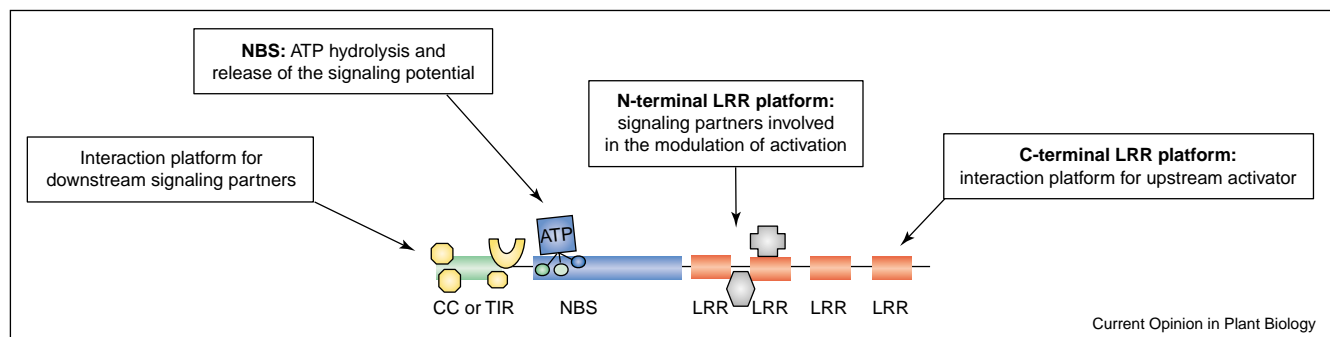
pre-formed and to stay largely in place upon activation [11,12]. Most of our discussion surrounds NBS–LRR protein activation by the type-III effector proteins that are delivered directly into the plant cell by phytopathogenic bacteria [8].

### A suit tailored for the job

Landmark progress in the past 10 years has resulted in the identification of functional *R* genes from model and crop species. The complete *Arabidopsis* genome sequence provides a detailed view of the structure of *R* gene loci [13]. The overwhelming majority of *R* genes encode NBS–LRR proteins [14<sup>\*</sup>]. This protein family is related to the mammalian caspase recruitment domain (CARD)/nucleotide-binding oligomerization domain (Nod) family, which also functions in innate immunity [15<sup>\*\*</sup>,16–18].

Each NBS–LRR protein (Figure 1) encodes a conserved NBS for ATP binding and hydrolysis [19]. A domain that includes the NBS can be enlarged by homology between R proteins and some eukaryotic cell-death effectors such as Apoptotic protease activating factor-1 (Apaf-1) and cell death protein-3 (Ced3) [16,17]. Their most striking structural feature is a variable number of carboxy-terminal LRRs. In many cases, the activation of downstream effectors of the mammalian CARD/Nod proteins is architecturally organized as follows: the amino-terminus is required for protein–protein interactions with an adaptor protein, whereas the NBS domain is responsible for ATP hydrolysis and release of the signal. The amino-terminal and carboxy-terminal domains within the LRRs appear to have different functions. The amino-terminal domain of the LRR appears to modulate activation, whereas specific residues that are located in the carboxy-terminal domain of the LRR appear to be responsible for bacterial

Figure 1



Domain structure of NBS–LRR proteins. A schematic representation of NBS–LRR proteins shows a domain-based platform for the assembly of various putative regulatory factors (i.e. CC, TIR, NBS and amino-terminal [N-terminal] LRR regions) necessary for controlled signaling. These domains also link to a possible intramolecular regulatory region on the carboxy-terminal (C-terminal) LRR. The cartoons in yellow represent putative interactors assembled on and carboxyl to the CC/TIR domains. The blue square represents ATP, but could also be GTP. The gray cartoons that are associated with the amino-terminal part of the LRR domain represent another set of putative interactors that might be positive regulators, as predicted from studies of the *rps5.1* allele [45]. Note that one of the yellow shapes might represent RIN4, as its interaction with RPM1 was mapped to the first 176 amino acids extending from the amino-terminus to just before the NBS.

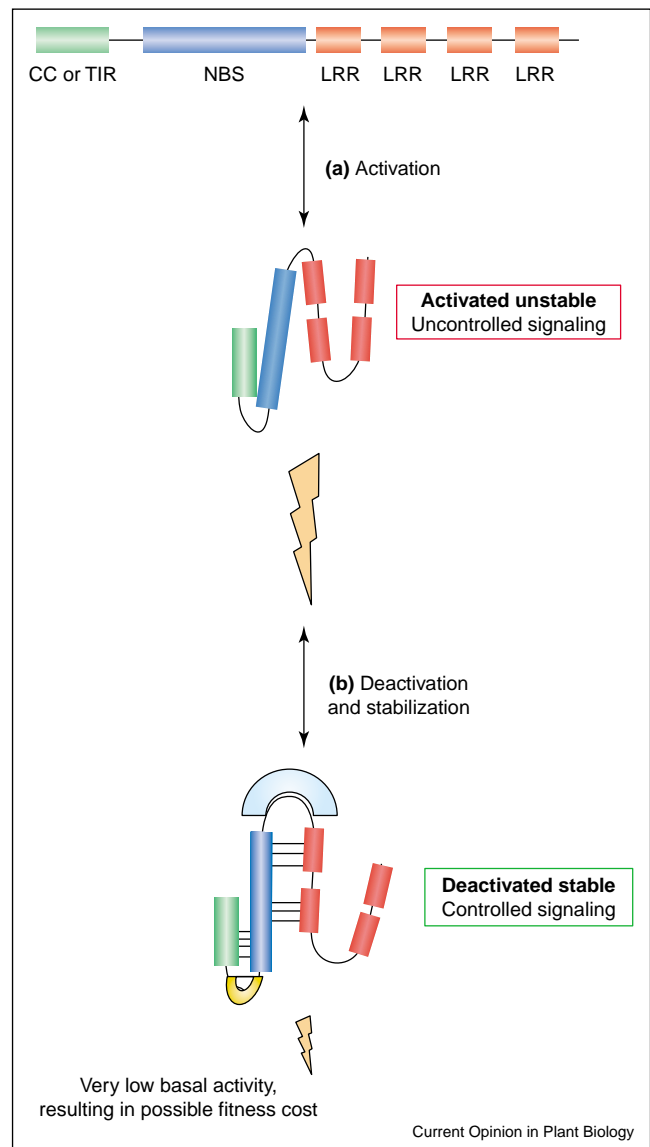
recognition, and hence define this region of the LRR as an interaction platform for upstream activators [16,20<sup>••</sup>,21]. These studies gave rise to a model for Apaf-1 activation called the 'jack-knife' model [22<sup>••</sup>].

### Do intra- or inter-molecular interactions negatively regulate NBS-LRR signaling?

Several reports indicate that NBS-LRR proteins are subject to constitutive negative regulation ([23–26], reviewed in [27<sup>•</sup>]; Figures 2 and 3). In at least some cases [22<sup>••</sup>], intramolecular interactions between R-protein domains may function as an on/off switch (depicted in Figure 2). The coiled-coil (CC)/*Drosophila* Toll and mammalian Interleukin 1 receptor (TIR), the NBS and the LRR domains appear to be critical for this process. Different types of mutation (either deletion or mis-sense mutation) that affect the integrity of these domains can result in gain-of-function mutation [28,29] that are often deleterious [18]. An elegant structure–function study of the potato NBS-LRR protein Rx (which confers resistance to Potato Virus X [PVX]) suggested how an NBS-LRR protein might manage its signaling potential via intramolecular negative regulation ([22<sup>••</sup>]; Figure 2). Moffett *et al.* [22<sup>••</sup>] demonstrated that physical interactions occur *in vivo* between the NBS-LRR domain and the amino-terminal CC. In addition, interactions can occur between the LRR and the CC-NBS [22<sup>••</sup>]. Importantly, these intramolecular interactions were disrupted in the presence of the relevant avirulence protein, in this case the PVX coat protein (CP). Moffett *et al.* [22<sup>••</sup>] proposed a model in which CP activates Rx by relieving negative regulation of the NBS and/or by inducing the recruitment of positive effectors. Gain-of-function mutations in Rx that lead to constitutive cell-death signaling encode changes in the LRR or in conserved motifs of the NBS [23]. However, analyses of possible intramolecular interactions between mutated domains of Rx, using the strategy described by Moffett *et al.* [22<sup>••</sup>], have not yet been reported. These might provide insight as to how Rx stores and holds its signaling capacity, and as how this protein is activated following the recognition of the viral coat protein.

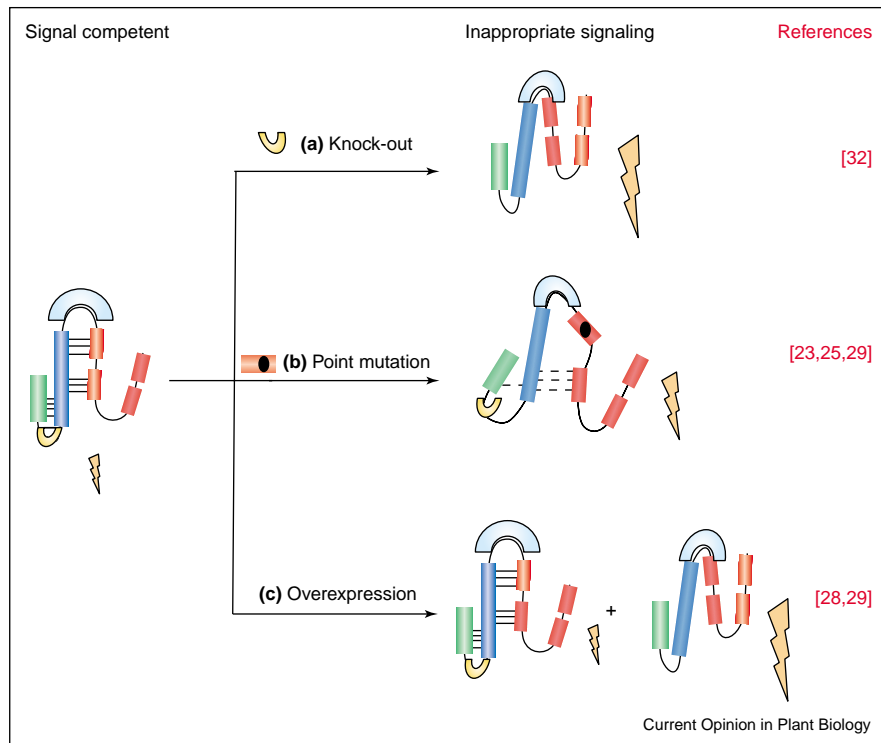
As underscored by Moffett *et al.* [22<sup>••</sup>], indirect interactions have not been ruled out in these events. It could be that the results described above are the effect of bridging or intermolecular interactions upon a scaffold. Indeed, several lines of evidence suggest that negative regulatory events that occur during NBS-LRR activation can be provided by interactions with *trans* partners. Overexpression of a single signal component can lead to the activation of the pathway in which it functions. For example, overexpression of NOD2 in mammalian cells enhances NF- $\kappa$ B activation [15<sup>••</sup>]. In addition, overexpression of the NBS-LRR proteins Rx, RPM1, RPS2, and *Pseudomonas* RESISTANCE AND FENTHION SENSITIVITY (PRF) induces cell death *in planta* in the absence of

Figure 2



Model for the generation of NBS-LRR protein signaling potential by *cis* and *trans* events. The signaling competency of NBS-LRR proteins is potentially governed at two levels. (a) In the absence of negative regulators, an activation event happens after the arrangement of the tertiary-quaternary structure. NBS-LRR proteins are dynamic and can be inappropriately activated (represented by the lightning bolt) and possibly unstable. Inappropriate activation has a deleterious effect on the cell, but (b) deactivation/stabilization is mediated by negative regulators (yellow block arc) and quaternary molding proteins (light blue block arc), which establish a stable, signal-competent conformation. In this cartoon, the negative regulator modulates the activated conformation of the CC-TIR-NBS domain by stabilizing intramolecular contacts with the LRR region. Note that the lightning bolt is still present because at least some NBS-LRR proteins probably have basal activity. This basal activity is not overtly deleterious but possibly incurs a fitness cost [47]. The postulated scenario is probably a transient event and could happen at either a co- or a posttranslational level.

Figure 3



Hypothetical mechanisms for inappropriate activation of NBS-LRR proteins. Three scenarios to describe how an NBS-LRR protein might be inappropriately activated. **(a)** Inappropriate activation of an NBS-LRR protein caused by genetic elimination of a negative regulator. **(b)** Inappropriate activation due to mutation(s) in the LRRs. These mutations presumably disrupt the interaction between the LRR and the NBS, as well as potential interactions between the NBS and the CC-TIR domain. This new conformation results in moderate activation (indicated by the size of the lightning bolts) because the negative regulator is unable to prevent this inappropriate activation (Figure 2). Missense mutations in the NBS or the CC-TIR domain that result in constitutive activation could also be represented by this model. **(c)** If the NBS-LRR protein were overexpressed, negative regulators might eventually be titrated out, resulting in inappropriate activation of an NBS-LRR protein due to overexpression and titration of a negative regulator.

pathogen. In fact, overexpression of the amino-terminus and NBS domains of RPS2 are sufficient to drive constitutive cell death, a phenomenon termed the 'overdrive' effect [28]. These overdose phenomena potentially emphasize another level of fine-tuned regulation between NBS-LRR-proteins and *trans* partners.

### Are NBS-LRR proteins regulated by their partners in the absence of stimulus?

A simple model posits that NBS-LRR are normally held in check by negative regulatory adaptors (Figures 2 and 3). Thus, inappropriate NBS-LRR activation would be correlated to the level of NBS-LRR accumulation. In contrast, positive pathogen-dependent signaling would merely reflect the modification of a finely tuned negative regulation, and subsequent NBS activation would be a default. It is thus plausible that manipulation of NBS-LRR stability, both in *cis* and in *trans*, could be a sufficient regulatory mechanism to ensure both rapid response and a minimum of inappropriate activation. Below, we discuss two possible negative regulatory mechanisms, deactiva-

tion and stabilization, using recently defined NBS-LRR protein partners as models.

### A model of the negative regulation of NBS-LRR proteins by *trans* partners

RIN4 is an evolutionarily conserved protein of 211 amino acids, the sequence of which provides no clues to its function. RIN4 interacts physically with the NBS-LRR proteins RPM1 and RPS2 *in vivo* at the plasma membrane [30<sup>••</sup>,31<sup>•</sup>,32<sup>•</sup>]. RIN4 is required for RPM1-mediated disease resistance, and is phosphorylated upon infection with *P. syringae* expressing the type-III effectors AvrB or AvrRpm1 [30<sup>••</sup>]. AvrRpt2, a sequence-unrelated type-III effector that is a cysteine protease, causes (either directly or indirectly) the posttranscriptional disappearance of RIN4. Overexpression of RIN4 blocks both AvrRpt2-dependent RIN4 disappearance and RPS2 activation. It is therefore likely that the disappearance of RIN4 causes the activation of RPS2-mediated disease resistance [31<sup>•</sup>,32<sup>•</sup>]. Despite the overwhelming evidence that RIN4 is a target for AvrB, AvrRpm1 and AvrRpt2, it is

apparently not the only cellular target for the virulence functions mediated by these type-III effectors (Y Belkhadir, D Mackey, JL Dangl, unpublished). Thus, pathogens may deploy virulence factors that target several host proteins. Polymorphism in, or absence of, these host targets will not always lead to a concomitant diminution of the effects of a single virulence factor during infection.

*rin4* null mutants are lethal in an *RPM1 RPS2* background [31<sup>•</sup>]; and this lethality is largely, but not completely, reversed in a *rin4 rps2* double mutant [31<sup>•</sup>]. These *rin4 rps2* plants remain slightly activated for defense gene expression. The lethal phenotype is fully eliminated in the *rin4 rps2 rpm1* triple mutant (Y Belkhadir, D Mackey, JL Dangl, unpublished), however, indicating that the mis-regulation of defense genes in *rin4* plants is dependent on inappropriate activation of both *RPS2* and *RPM1* in the absence of pathogen. Thus, RIN4 negatively regulates the inappropriate activation of both of these NBS-LRR proteins. We propose that binding of RPM1 and RPS2 to RIN4 may keep these proteins in an 'off' yet signal-competent configuration.

It is legitimate to question whether the normal function of RIN4 is to negatively regulate RPM1 and RPS2. We favor this idea because *rin4 rps2 rpm1* triple mutants have no other observable phenotypes under normal growth conditions. It remains to be determined whether the inappropriate activation of RPS2 and RPM1 observed in *rin4* null plants is mechanistically distinct from the appropriate type-III-effector-specific activation of these two NBS-LRR proteins following infection. Furthermore, there are apparently no other NBS-LRR proteins that are negatively regulated by RIN4. There are, however, several other small proteins in *Arabidopsis* that share a short domain with RIN4. They may act as adaptors and/or negative regulators of other NBS-LRR proteins. Adaptors without enzymatic function can be central mediators of signal transduction in animal immunity [33<sup>••</sup>]. For example, adaptors can be both positive and negative regulators of T-cell signaling [33<sup>••</sup>]. Adaptor null mutations can lead to the complete absence of some cell lineages [33<sup>••</sup>] that normally require up- or downregulation of receptor activity for their determination.

A second example of a protein that negatively regulates an NBS-LRR protein was described recently [34<sup>•</sup>]. *SNC1* encodes a TIR-NBS-LRR protein that is a member of the RPP4 and RPP5 protein family [26]. This protein was isolated in an *Arabidopsis* genetic screen for suppressors of the *non-expresser of PR-genes1-1* (*npr1-1*) mutation [35]. The original *npr1-1* mutant does not exhibit defense gene activation or systemic acquired resistance in response to salicylic acid. The *snc1* suppressor mutation results in constitutive defense gene expression and disease resistance [26]. This mutation is a missense mutation that is located between the NBS domain and the LRRs. The

*BONZAI1* (*BON1*) gene is required for *Arabidopsis* growth homeostasis at varying temperatures [36]. In essence, the *bon1-1* mutant is a temperature-sensitive lesion mimic, which is dwarfed and activated for defense responses and which resembles a *rin4* knock-down anti-sense line. SNC1 activity is responsible for this phenotype, as a *bon1-1 snc1* double mutant resembles the wildtype when grown at 22 °C [34<sup>•</sup>]. Therefore, *BON1* is a negative regulator of temperature-dependent SNC1 activation. Interestingly the BON1 protein is a phospholipid-binding protein that is associated with the plasma membrane [36]. *In vitro*, *BON1* is able to enhance vesicle aggregation. BON1 appears to work hand-in-hand with its interactor BAP1, as overexpression of *BAP1* partially suppresses the *bon1-1* temperature-dependent phenotype [36]. Whether BON1 and BAP1 reside in a multiprotein complex that is guarded by SNC1, and whether BON1 or/and BAP1 are targeted by any pathogen or activated by any infection, remains to be determined.

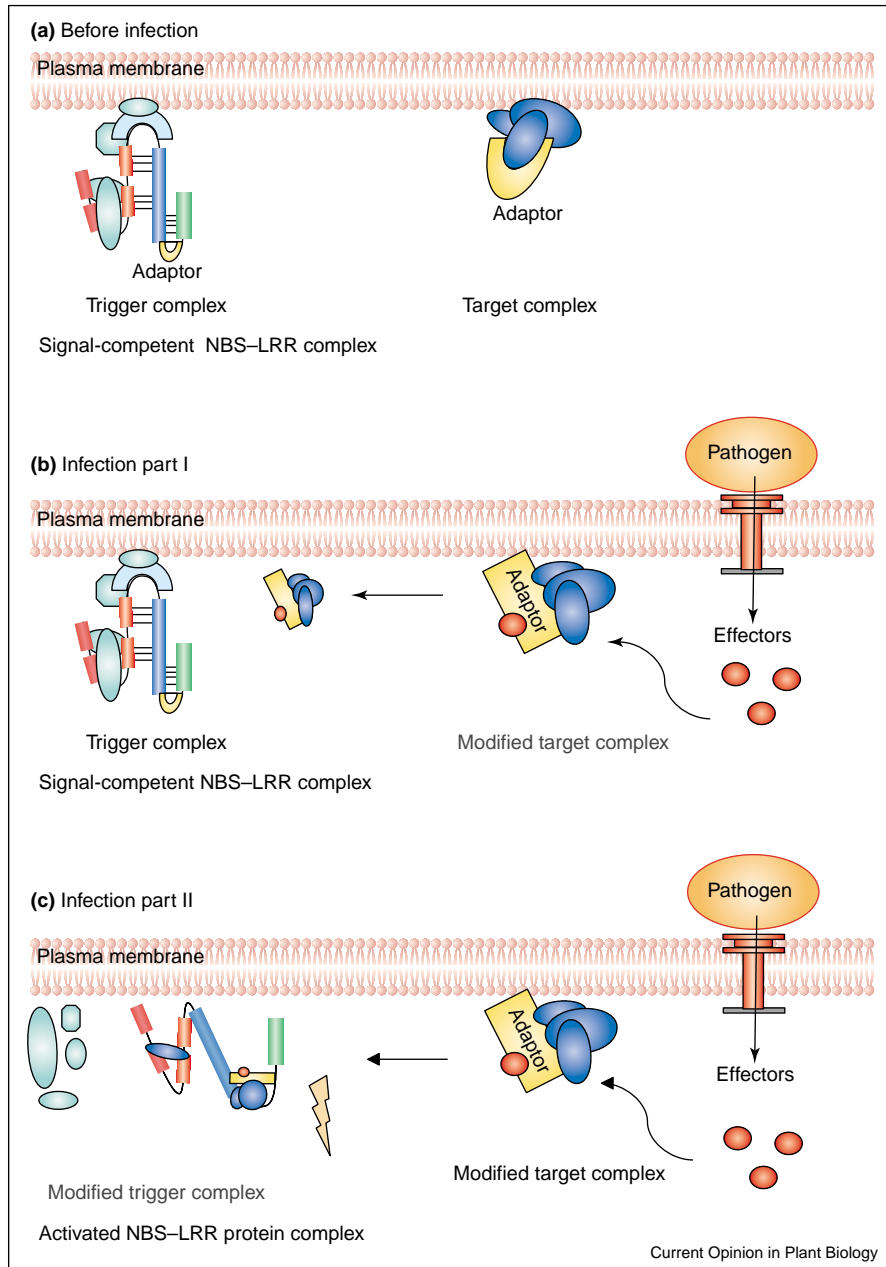
### A model for NBS-LRR stabilization by *trans* partners

Recent data suggest that NBS-LRR *trans* partners might be required to mold and maintain intra-molecular interactions that are required for signal competence and/or to mediate indirect recognition during infection. Cytosolic HSP90s are required for some NBS-LRR functions and interact *in vivo* with some NBS-LRR proteins (e.g. RPM1 [37<sup>••</sup>], RPS2 [37<sup>••</sup>], the tobacco N protein [38<sup>•</sup>], and presumably the potato Rx protein [39<sup>•</sup>]; reviewed in [40]). *Arabidopsis* contains four cytosolic HSP90s. Using an extensive forward-genetic approach Hubert *et al.* [37<sup>••</sup>] uncovered three point mutations in the *Arabidopsis HSP90-2* gene that specifically impair RPM1 signaling. These rare and unique mutations are clustered in the highly conserved amino-terminal ATPase domain, and probably generate a mutant HSP90 that poisons RPM1 signaling specifically (as other NBS-LRR functions are not affected by these mutations). Strikingly, an *hsp90-2* knockout did not affect RPM1 function, suggesting that the absence of HSP90-2 can be functionally complemented by one of the other three cytosolic HSP90s. This is consistent with results from yeast [41]. Similarly, the gene silencing of individual HSP90 isoforms in *Nicotiana benthamiana* had no effect on Rx function, whereas general silencing of all HSP90 isoforms did [39<sup>•</sup>]. It is therefore surprising that Takahashi *et al.* [42<sup>•</sup>] suggest that an *hsp90-1* knockout alters RPS2 function (but not other tested NBS-LRR functions). The *hsp90-2* missense mutants exhibit significantly decreased RPM1 levels [37<sup>••</sup>], and HSP90 silencing in *N. benthamiana* reduces Rx accumulation [39<sup>•</sup>]. Therefore, RPS2 levels should be significantly lowered in the *hsp90-1* knock out. Accordingly, RPM1 levels should not be affected in the *hsp90-2* knockout. We eagerly await the measurement of RPS2 and RPM1 accumulation in the *hsp90-1* and *hsp90-2* knockouts.

Do other NBS-LRR proteins use the same mode of regulation? We envisage that a selective requirement for HSP90 for some, but not all, NBS-LRR functions will depend on the inherent stability of the NBS-LRR protein

before and during activation. Given the recent models for the function of cytosolic HSP90 in molding and holding activation-competent signal complexes in a variety of cellular contexts [43], we suggest that HSP90s might work

Figure 4



A possible soft-wired signal in NBS-LRR activation. **(a)** We propose that two different protein complexes are present at the plasma membrane before pathogen elicitation. The ‘trigger’ complex contains an NBS-LRR protein associated with an adaptor (yellow block arc) that, together with downstream signaling proteins (light blue shapes), is responsible for its negative regulation. The black arrows indicate a dynamic event triggered by pathogen effectors. The ‘target’ complex contains the same adaptor molecule and putative signaling components. Note the stoichiometric differences between the trigger complex and the ‘target’ complex. **(b)** After infection, the pathogen effectors (red circles) associate with the target and modify the adaptor protein. A small proportion of the modified complex then associates with the trigger complex. **(c)** The association of the modified target complex with the trigger complex results in the displacement of the negative regulator and other downstream signaling molecules. At this point, the trigger complex undergoes conformational changes and becomes poised for action. This can result in the recruitment of new partners and re-localization to other cellular compartments.

hand-in-hand with proteins that are implicated in NBS-LRR stability, such as SGT1b and RAR1, to achieve this selectivity [40]. Such fine-tuning of NBS-LRR stability should reflect, first, the requirement for rapid activation upon appropriate stimulation, and second, the need to minimize inappropriate activation that, in studies carried out to date, invariably leads to decreased cellular viability.

### NBS-LRR regulation in the presence of virulence factors

RIN4 is targeted by three different type-III effectors and negatively regulates two distinct *Arabidopsis* NBS-LRR proteins, fulfilling two important corollaries of the guard hypothesis [9,10]. An even more explicit example of indirect NBS-LRR activation was provided recently [44\*\*]. RPS5 is an NBS-LRR protein that recognizes bacteria that express the type-III effector AvrPphB [45]. This recognition requires PBS1, a serine-threonine kinase. Strikingly, PBS1 is a direct substrate of the cysteine protease type-III effector AvrPphB, which cleaves it once [44\*\*]. AvrPphB appears to associate *in vivo* with full-length PBS1 and also with the carboxy-terminal cleaved product. Shao *et al.* [44\*\*] demonstrated that PBS1 cleavage and kinase activity were genetically upstream of, and independently required for, the activation of RPS5-mediated resistance [44\*\*]. Thus, AvrPphB is recognized via the production of PBS1 cleavage products, rather than through a simple and direct interaction with RPS5 [44\*\*]. The roles of the cleavage products in AvrPphB virulence activities and RPS5-induced signaling have not yet been determined.

The isolation and identification of NBS-LRR partners by biochemical purification is likely to be the next challenge in this field. Preliminary biochemical studies of the Mildew resistance locus A protein (MLA1) from barley indicate that this 100-kDa protein is part of a 700–800-kDa protein complex [46]. Using native gel filtration, before infection, RPM1 and RPS2 (proteins of about 110 kDa) elute in fractions of approximately 1500 kDa in our buffer conditions (R Subramaniam, Y Belkhadir, JL Dangl, unpublished), suggesting the existence of a multi-protein complex. As expected from co-immunoprecipitation data [30\*\*,31\*,32\*], a small amount of the 24-kDa RIN4 protein co-elutes with RPM1. The majority of RIN4, however, is in an RPM1/RPS2 free 200-kDa fraction. Following pathogen challenge, these two pools of RIN4 are dynamic (Figure 4). RPM1 redistributes into fractions ranging from 440–1500 kDa. The smaller complexes may represent ‘activated’ RPM1 protein complexes that contain different protein partners. Whether or not the RIN4 dynamic that we observe is responsible for bringing new partners to the RPM1-containing fractions remains to be determined. This dynamism is consistent with the role of RIN4 as an adaptor, however, as adaptors are often crucial for bringing effectors into close proximity to their targets [33\*\*].

### Soft-wired or hard-wired NBS-LRR signaling

In the light of the guard hypothesis, the interactions of RPS2/RPM1/RIN4 argue for ‘soft-wired NBS-LRR protein signaling’ in which signaling molecules are modified, and mobilized into and out of a receptor-containing complex, after stimulation by the upstream effector. In this model, the receptor itself might be subjected to dynamic events, including re-localization, new effective interactions, and posttranslational modifications after activation.

The converse ‘hard-wired NBS-LRR protein signaling’ model is not mutually exclusive, but it is perhaps less sophisticated. Hard-wired signaling predicts that all of the required components are already assembled, and that a possible direct interaction with a pathogen effector triggers the activation of the complex, the subsequent release of downstream effectors, and the possible diffusion of second messengers. Hard wiring represents signaling events that are mechanistically and architecturally rigid. Such events might lack the latent plasticity that is presumably necessary for sharing common signaling components in potentially varied sub-cellular compartments [45], for regulating responses to multiple stimuli that might be generated by pathogens that deploy multiple virulence factors during infection [8], and for driving new interactions with the cellular targets of pathogen virulence factors.

### Conclusions

Tremendous progress has been made in improving our understanding of the plant immune system using well-tailored genetic approaches. These approaches are reaching saturation, however, and have not revealed the mechanistic and physiological basis of NBS-LRR protein action. This understanding will come with the development in parallel of, first, sophisticated biochemical approaches that are focused on the biology of adaptor and effector target proteins, and second, cellular and imaging approaches that enable the real-time analysis of resting and activated NBS-LRR signaling complexes.

### Acknowledgements

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