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STANDing strong, resistance proteins instigators of plant defence

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Resistance (R) proteins are involved in specific pathogen recognition and subsequent initiation of host defence. Most R proteins are nucleotide binding – leucine rich repeat (NB–LRR) proteins, which form a subgroup within the STAND (signal transduction ATPases with numerous domains) family. Activity of these multi-domain proteins depends on their ability to bind and hydrolyse nucleotides. Since R protein activation often triggers cell-death tight regulation of activation is essential. Autoinhibition, which seems to be accomplished by intramolecular interactions between the various domains, is important to retain R proteins inactive. This review summarizes recent data on intra- and intermolecular interactions that support a model in which pathogen perception triggers a series of conformational changes, allowing the newly exposed NB domain to interact with downstream signalling partners and activate defence signalling.

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Introduction

The long history of battle between pathogens and plants is evident by the sophisticated, multilayered immune system of plants [1]. The first line of the innate defence system is based on recognition of conserved pathogen-derived molecules, called MAMPs (microbe-associated molecular patterns), by pattern recognition receptors (PRR). Specialized microbes, however, can evade or suppress this MAMP triggered immunity (MTI) by secretion of virulence factors, so-called effectors. A subset of these effectors, referred to as AVR, can be perceived by resistance (R) proteins that trigger a second layer of host defence, referred to as effector triggered immunity (ETI). Rapid ion fluxes, an oxidative burst, and transcriptional reprogramming are induced during both MTI and ETI. Only with the

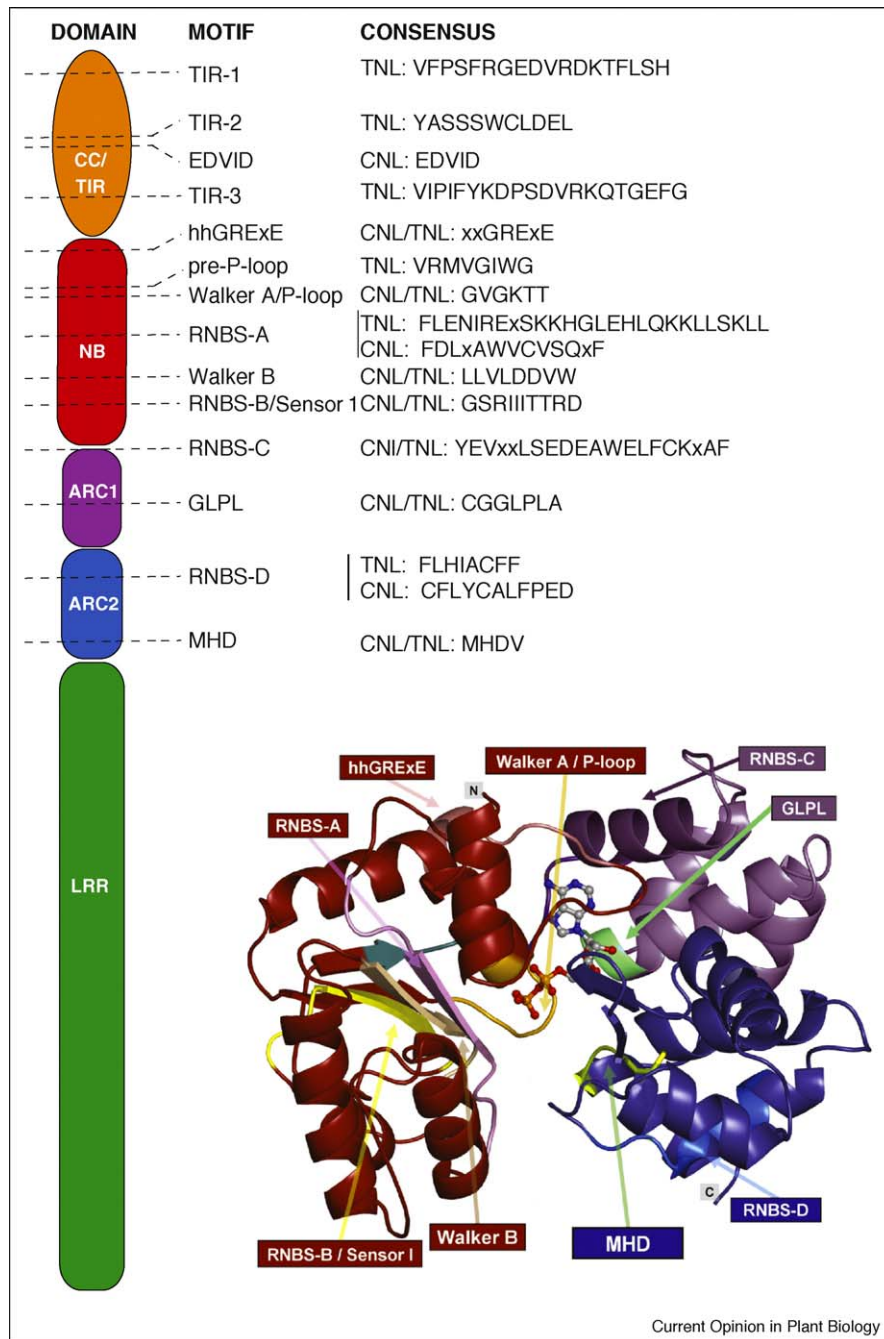
latter often also programmed cell death around the infection site occurs, which is called the hypersensitive response (HR).

The complex relation between plant resistance and pathogen virulence through co-evolution was recently described as a ‘zig-zag’ model [1]. An illustrative example of this zig-zag model is the interaction between tomato and *Fusarium oxysporum*. The fungus requires the effector AVR3 for full virulence, possibly to suppress MTI. This effector, however, can be recognized by the tomato R protein I-3 that subsequently triggers ETI. To counteract this defence response, it has been hypothesized that the fungus evolved a second effector (AVR1) that suppresses these I-3 mediated defences. To thwart the fungus the plant in turn evolved R protein I-1 that recognizes AVR1 and activates host defences once more [2*].

Over 40 R genes have been cloned over the last two decades and the majority belongs to the NB–LRR family, as they contain a nucleotide-binding domain (NB) fused to a C-terminal leucine-rich repeat (LRR) domain (Figure 1). The LRR domain is proposed to adopt an arc-shaped conformation, forming a protein-protein interaction surface [3]. The NB is part of a larger domain that is called the NB–ARC as it is shared between R proteins and the human apoptotic protease-activating factor 1 (APAF-1) and its *Caenorhabditis elegans* homolog CED-4. As indicated in Figure 1, many conserved motifs can be discerned in the NB–ARC domain. Proteins carrying an NB–ARC domain belong to the STAND (signal transduction ATPases with numerous domains) family of NTPases [4].

Based on 3D modelling, the NB–ARC of R proteins is proposed to contain three subdomains: the NB forming a P-loop NTPase fold, the ARC1 consisting of a four-helix bundle and the ARC2 adopting a winged-helix fold (reviewed in [5]; Figure 1). Most of the conserved motifs in the NB–ARC are present at the interface of these three domains where they form the nucleotide-binding pocket (Figure 1). The N-termini of NB–LRRs are structurally diverse. Some carry a domain having homology to the toll and human interleukin-1 receptor (TIR) domain and these R proteins are called TIR–NB–LRRs or TNLs. Non-TIR NB–LRR members are referred to as CC–NB–LRRs or CNLs, because many of them contain a predicted coiled coil region (CC), sometimes extended by a DNA binding domain such as a BEAF/DREAF zinc finger domain (BED) or by a solanaceous domain (SD) (reviewed in [6]).

Figure 1



Schematic representation of a typical NB–LRR protein. The (sub)domains are depicted as coloured boxes: CC/TIR domain (orange), NB (red), ARC1 (purple), ARC2 (blue) subdomains and LRR domain (green) whereas conserved motifs are marked as lines. Consensus of sequences is written next to name of the motif (according to [47]). Insert, predicted 3D structure of the NB–ARC domain of I-2 modelled on the ADP-bound Apaf1 (1z6t) template. Conserved motifs and N- and C-termini are marked. ADP and Mg atoms are depicted as balls and sticks (adopted from [35]).

Some NB–LRR proteins have been shown to bind to their cognate AVRs directly, whereas others have been shown to interact indirectly through an intermediary host-factor [7]. Such a host-factor could either represent a virulence target of the effector (guard model) [8] or a target mimic

(decoy model) [9]. In both models, modification of the host target by the effector triggers defence in resistant plants. However, only when the target is a guardee its manipulation by the effector enhances disease development in susceptible plants [9]. Either way, defence

signalling is activated by the R protein after AVR recognition whereby the R protein acts as a molecular switch. Here, we will focus on changes in intra- and intermolecular interactions of R proteins during their activation. As reversible, dynamic intramolecular interactions have been most intensively studied for the potato resistance protein Rx, we use this protein as a model. Based on Rx, we propose a mechanistic model for R protein activation that is then evaluated with data available for other R proteins and their interacting partners.

Intramolecular interactions in NB-LRR R proteins

R protein activation often triggers a hypersensitive response (HR). To prevent damage due to spontaneous inappropriate activation, these proteins must be under tight negative control. This seems to be accomplished by intramolecular interactions between the various subdomains of the R protein. Pathogen perception is proposed to release this autoinhibition, enabling the conformational changes required to activate defence signalling.

Intramolecular interactions in Rx

Rx is a CNL that confers resistance to Potato Virus X by recognition of the viral coat protein (CP). The LRR domain of Rx confers negative regulation, as its deletion results in a weak auto-activation phenotype [10]. However, the LRR domain also provides positive control, as expression of just the N-terminal half of Rx containing auto-activation mutations in the NB-ARC domain does not induce a strong HR unless both halves are co-expressed [11,12[•]]. Domain swaps between Rx and its paralogue Gpa2 revealed that pathogen recognition specificity is mainly mediated by the C-terminal part of the LRR. Furthermore, when C- and N-terminal parts of the LRR domain were combined with the ARC2 subdomain of its paralogue in swap constructs, HR was induced in the absence of a pathogen [12[•]]. Apparently, when incompatible domains are combined in one protein, autoinhibition is diminished. Autoactivation also resulted from specific point mutations that map either in the NB, the ARC2 or the N-terminal part of the LRR domain [10], indicating that these three subdomains are involved in autoinhibition and/or relaying pathogen recognition into signalling.

Surprisingly, CP-dependent HR can be reconstituted when the CC domain is co-expressed *in trans* with the NB-ARC-LRR domain, or the LRR domain *in trans* with the CC-NB-ARC. The three Rx domains physically interact, as shown by co-immunoprecipitation experiments [11], and the ARC1 was identified as the main scaffolding domain for LRR binding [12[•]]. This physical interaction between LRR and CC-NB-ARC is disrupted in the presence of the CP, but remained unaffected by point mutations in the NB-ARC or LRR domain that resulted in autoactivation or loss-of-

function [11,12[•]]. These observations imply that many residues scattered over the various domains may contribute to their intramolecular interaction and that CP perception might generate a more drastic conformational change than induced by autoactivating mutations. Notably, the NB-ARC domain alone does not suffice for LRR binding and the CC is required for a stable interaction [12[•],13^{••}].

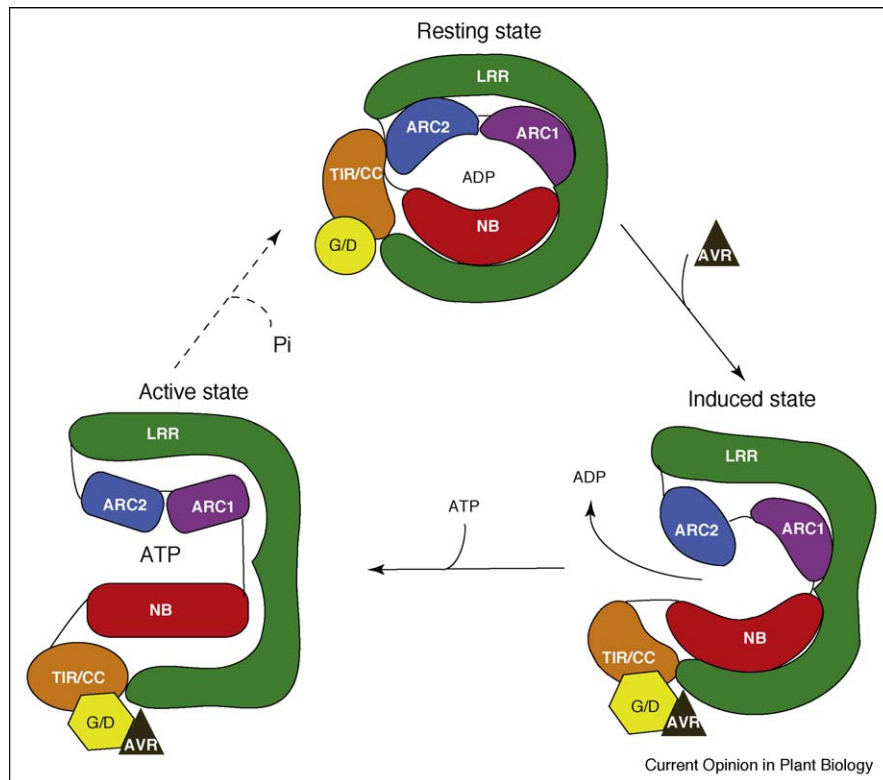
Conversely, the CC also does not bind the NB-ARC unless the latter is fused to the LRR. This CC-NB-ARC-LRR interaction is disrupted by the CP. But unlike the CC-NB-ARC-LRR interaction, it is also diminished by autoactivation or loss-of-function mutations in the NB-ARC domain [11,13^{••}]. Furthermore, this interaction can be abolished by single point mutations in the conserved 'EDVID' motif in the CC (Figure 1), suggestive for a defined interaction interface. The EDVID motif is part of a larger region that is responsible for the interaction with RanGAP2 (Ran GTPase Activating Protein 2) [13^{••},14,15]. Silencing RanGAP2 suppresses Rx-mediated PVX resistance, showing that it is required for Rx function. RanGAPs is known to regulate the activity of the GTPase Ran that controls nucleo-cytoplasmic trafficking [16]. How RanGAP affects Rx activity has yet to be determined.

A refined model for resistance protein activation based on Rx

Combining the current models on R protein function [5,17] with the recent data on Rx [13^{••}] allows us to propose a more refined model describing the interaction dynamics and specific functions of the individual subdomains in signalling. No nucleotide binding studies have been reported for Rx specifically, but the highly conserved NB-ARC domain of NB-LRRs appears to function as a molecular switch wherein the ADP bound state represents the 'off' and the ATP the 'on' state [5].

In our updated model, the CC and LRR both bind the NB-ARC, thereby providing an interaction platform that mediates CP recognition at their interface (Figure 2). Such a closed conformation of Rx would be stabilized by the N-terminal part of the LRR domain that negatively regulates R protein activity [10,11]. Currently, it is unclear how Rx senses the CP, co-immunoprecipitation experiments aiming to show an interaction between CP and RanGAP2 have been inconclusive [14,15]. Nevertheless, recognition of the CP releases autoinhibition conferred by the N-terminal part of the LRR allowing the NB-ARC domain to exchange ADP for ATP. ATP-binding by the NB-ARC abrogates its CC binding, which in turn affects the NB-ARC interaction with the C-terminal LRR domain. In this activated conformation the NB-ARC domain becomes exposed [11], allowing the protein to bind and activate downstream signalling

Figure 2



Model for NB-LRR protein activation. In the absence of a pathogen an NB-LRR protein resides in its resting (ADP) state, in which the LRR stabilizes the closed conformation. The recognition platform for the AVR protein (brown triangle) is provided by the C-terminal part of the LRR together with CC/TIR domain (CC) and the latter could be bound to an interactant (referred to as guard or decoy - G/D). Perception of the AVR (direct or via the G/D) changes the interaction surface between the N-terminal part of the LRR and the ARC2 subdomain, thereby releasing the autoinhibition conferred by the LRR. Subsequent nucleotide exchange triggers a second conformational change, altering the interactions of the NB-ARC domain with CC and LRR domains (induced state). In the activated state the NB subdomain is accessible to interact with downstream signalling partners. Hydrolysis of ATP could return the protein to its resting state.

partners. Possibly these functions are provided by the NB subdomain because it triggered HR in the absence of the CP [13]. Apparently, overexpression of NB subdomain overrules the endogenous requirements needed for Rx to trigger defence signalling. In conclusion, in this model pathogen perception triggers a series of conformational changes, allowing the exposed NB domain to trigger defence signalling.

Intramolecular interaction in other NB-LRR R proteins

Is the model proposed above also applicable to NB-LRR proteins other than Rx? Analogous to Rx, negative and/or positive regulatory functions for the LRR domain have been identified for RPS5, I-2, RPS2, RPS1A and Mi-1.2 [18–21]. Furthermore, genetic and molecular studies showed that, similar to Rx, recognition specificity is often provided by the LRR domain which is under diversifying selection and highly variable [3]. For the L proteins of flax, that confer resistance to the rust fungus *Melampsora lini*, the LRR has been proposed to be the major recog-

nition domain, although for a subset of these proteins the TIR domain has also been shown to play an important role in determining specificity [22]. However, in a yeast two-hybrid experiment, a specific interaction with the AVR protein only requires the NB-LRR domains of L and not the TIR domain [23].

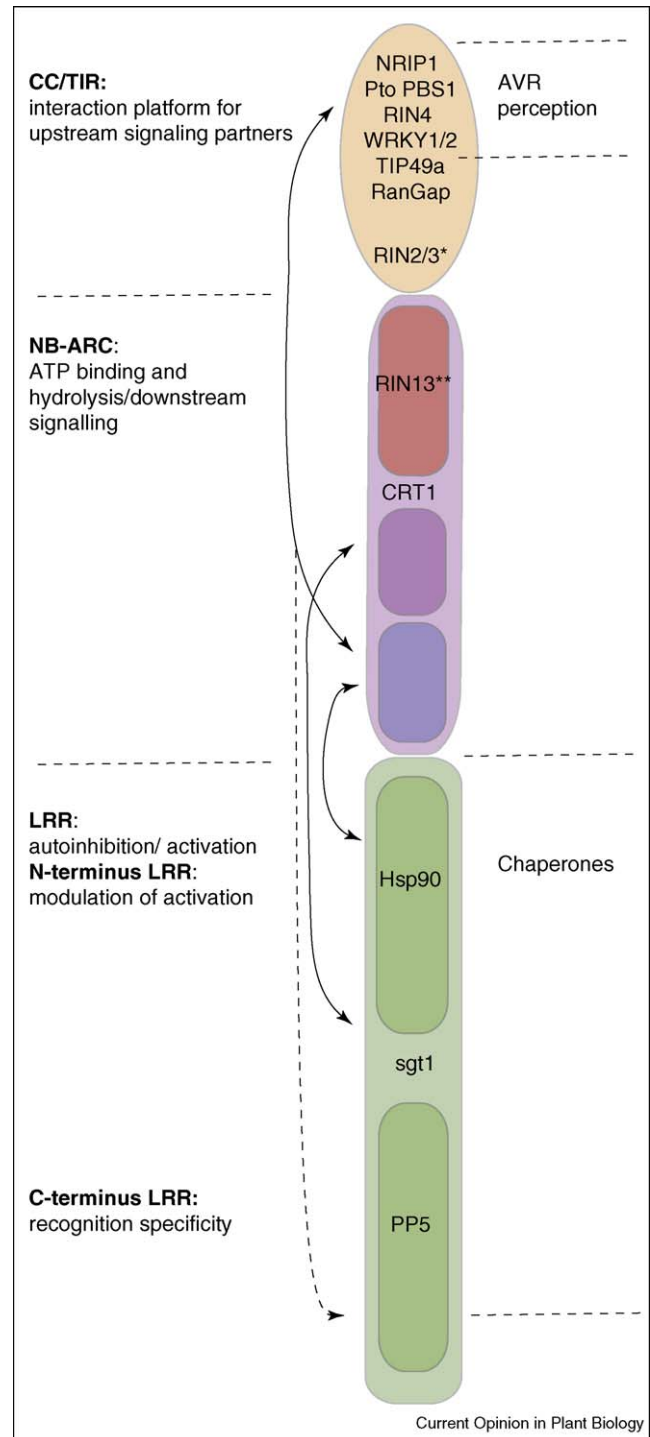
Physical interaction between the LRR domain and other R protein parts has been observed *in vivo* for Bs2, RPS5 and Mi-1.2 [18,19,24]. Only for Bs2 this interaction was analyzed in the presence of the cognate AVR protein. Unlike Rx, the interaction between CC-NB-ARC and LRR was not disrupted by the AVR protein. In Mi-1.2, similar to Rx, the interaction between CC-NB-ARC and LRR was found to be constitutive and independent of the presence of autoactivation or loss-of-function mutations in the NB-ARC domain [19].

Interaction between CC and NB-ARC domains has also been reported for RPS5 [18]. However, neither for Bs2 nor for N, a TNL protein, a physical interaction between

the N-terminus and remaining part could be detected *in vivo* [24,25]. An interaction, possibly a transient one, cannot be excluded, as the co-expression of the same Bs2 domains reconstitutes protein function, suggesting interaction. Taken together, although data for intramolecular interaction of NB-LRR proteins remain scarce, they already reveal some differences in how these proteins are regulated and function. Despite differences within one class, it will also be of interest to investigate whether there are general class-specific interaction patterns for CNL and TNL proteins. An interesting observation is that overexpression of the TIR domain of some TNL proteins (L10, RPS4, RPP1A, At4g19530) can trigger HR, but only when fused to a small part of the NB-domain containing the TIR-specific pre-P-loop motif [26,27]. Either the extended TIR domain is sufficient to interact and activate downstream signalling partners, or it might activate other TNLs *in trans*, thereby causing an HR phenotype. Immunoprecipitation studies using the extended TIR domain as bait may provide an answer to this question.

In the Rx model, both the LRR domain and the N-terminus are required for recognition whereas the NB subdomain is responsible for downstream signalling. How is this to be reconciled with the observation that TNLs and CNLs, having different N-termini, have a different requirement for downstream signalling components: EDS1/PAD4 for TNLs and NDR1 for CNLs [28]. Besides carrying structurally different N-termini, the NB-ARC domains of TNLs and CNLs show clear distinctions in two R protein specific NBS (RNBS) motifs: the RNBS-A and RNBS-D ([29,30]; Figure 1). The RNBS-A is part of the NB subdomain whereas the RNBS-D belongs to the ARC2 subdomain. Functional importance of both motifs is signified by the autoactivation and loss-of-function mutations observed in these motifs [10,31,32,33]. These conserved RNBS motifs are not found in the related STAND proteins belonging to the NACHT-LRR (NAIP, CHITA, HET-E, TP-1) family. Interestingly, however, NACHT proteins contain a conserved motif, called Motif-II, located at the position corresponding to the RNBS-A. This positional conservation implies that this motif could be important for interaction with distinct partners [34]. Support for this idea comes from 3D modelling studies. Using ADP-bound APAF-1 (resembles resting state) [35] and ATP-bound CED4 (mimics the activated state) (unpublished) as template, it can be observed that the RNBS-A motif is buried in APAF, but more exposed in CED-4, due to the translocation of the ARC2 domain, making it potentially available for an interacting partner. The RNBS-D is located on the ARC2 that in APAF-1 is involved in the interaction with the N-terminal CARD domain [36]. Solving the ATP-bound structure of activated APAF-1, or of an NB-LRR R protein, should determine whether accessibility to these two motifs indeed changes upon nucleotide exchange.

Figure 3



Schematic relation between intra- and intermolecular interactions found with NB-LRR domains. NB-LRR proteins consist of three major domains: CC/TIR (orange), NB-ARC (pink) and LRR (green). The NB-ARC domain (pink) can be subdivided into the NB (red), ARC1 (purple), ARC2 (blue) subdomains, and the LRR (green) is roughly divided into a C- and a N-terminal part. Intramolecular interactions between domains are indicated with double headed arrows. Proteins physically interacting with a specific (sub)domain are indicated at the corresponding region in the structure. RIN2/3* – also requires hhGREX motif in the NB. RIN13** – also requires C-terminal half of the CC domain.

Since the NB of Rx alone can trigger HR [13**], it is tempting to speculate that the RNBS-A in the NB might contribute to the observed specificity in downstream signalling components. The tobacco TNL protein N, conferring resistance to tobacco mosaic virus, provides independent support for a role of the RNBS-A motif in downstream signalling. Specific point mutations in the RNBS-A disrupt the protein's ability to induce viral resistance and to trigger HR, but did not alter its ability to oligomerize upon pathogen perception. Oligomerization, which requires a functional nucleotide-binding site, was suggested to be one of the earliest events in elicitor-mediated activation of N. Since RNBS-A mutations did not influence oligomerization, the RNBS-A could be involved in interaction with downstream signalling components [25]. Future experiments, in which the TNL- and CNL-specific motifs are swapped or mutated, can put this model to the test.

The role of the RNBS-D in the ARC2 is unknown, but the observed co-evolution of RNBS-D and the type of N-terminal domain suggests that it could be involved in the interaction between the ARC2 and the N-terminus in NB-LRR R proteins, in line with the proposed intramolecular interactions in Rx (Figure 2).

Intermolecular interactions of NB-LRR R proteins

R protein activity is also regulated by intermolecular interactions. Specifically, these interactions have been found to be required for R protein accumulation and/or pathogen recognition. Identification of interacting partners can provide insight into the specific roles of the different domains in R protein function (Figure 3). Table 1 lists proteins that physically, and genetically interact with NB-LRR R proteins. Most interactors have been identified by yeast two-hybrid screens, but recently

Table 1

Proteins physically and genetically interacting with NB-LRR R proteins.

Interactor	NB-LRR protein analyzed		Activity	Relation to R protein function	Ref.
	Interacting	Non-interacting			
NRIP1: N receptor interacting protein 1	N ^{a,b}	Bs4 ^{a,b}	Rhodanese sulfurtransferase	AVR perception: in the presence of p50 (AVR) NRIP1 translocates from the chloroplast and binds N	[48**]
Pto: resistance to <i>P. syringae</i> pv. tomato	Prf ^b		Kinase	AVR perception: binds AvrPto & AvrPtoB	[49]
PBS1: avrPphB susceptible	RPS5 ^b		Kinase	AVR perception: PBS1 is cleaved by AvrPphB	[18,50]
RIN4: RPM1 interacting protein 4	RPM1 ^{a,b} RPS2 ^b	RPP5 ^a	Unknown; required for basal resistance (repressor)	AVR perception: RIN4 is phosphorylated by AvrRpm1 and AvrB and cleaved by AvrRpt2	[51–53]
WRKY1/2: transcription factors	Mla10 ^{a,b}		Transcription factors; repressors of basal resistance	AVR perception: interacts with Mla10 in the presence of AVR _{A10}	[54**]
TIP49a: (RIN1) RPM1 interacting protein 1	RPP5 ^a RPM1 ^{a,b}	RPS2 ^a	Transcriptional regulator; interacts with the TATA-binding protein complex	Unknown	[55]
RanGap: RanGTPase-activating protein	Rx ^b Rx2 ^b Gpa2 ^b	BS2 HRT ^b Prf N ^b	Nucleo-cytoplasmic transport	Unknown	[14,15]
RIN2/3: RPM1 interacting protein 2/3	RPM1 ^{a,b} RPS2 ^a	RPP5 ^a	RING-finger E3 ligase	Unknown	[56]
RIN13: RPM1 interacting protein 13	RPM1 ^{a,b}		Unknown	Unknown	[42]
CRT1: compromised recognition of TCV	HRT1 ^b SSI4 ^b Rx ^b RPS2 ^b		ATPase activity; GHKL member	Unknown	[43*]
Sgt1: suppressor of G2 allele of skp1	Mla1 ^a Bs2 ^b	Mla6 ^a	Co-chaperone of Hsp90/ Hsp70; binds RAR1 member SCF complex	(co)Chaperone/ proteasome	[24,57]
Hsp90: heat shock protein 90	N ^{a,b} RPM1 ^b Mla1 ^a Mla6 ^a I-2 ^a		Chaperone; ATPase interacts with Sgt1, RAR1 and PP5	Chaperone	[57–60]
PP5: protein phosphatase 5	I-2 ^{a,b} RPM1 ^a Mi-1.2 ^a N ^a		Phosphatase; co-chaperone of Hsp90	(co)Chaperone	[58,60]

^a Identified using yeast two-hybrid.

^b Identified using co-immunoprecipitation.

also co-immunoprecipitation experiments have been successful.

As can be seen in Table 1, most interactors specifically bind one or a few closely related R proteins. The exceptions often interact with the LRR domain of NB-LRRs (Figure 3). These three LRR interactors, heat shock protein 90 (Hsp90), suppressor of G2 allele of *skp1* (Sgt1) and protein phosphatase 5 (PP5), have all been proposed to act as molecular (co)chaperones and interact with a diverse set of R proteins (see references in Table 1). Chaperones facilitate protein folding and stability under changing environmental conditions that could otherwise lead to their aggregation [37]. Sgt1 was found to control the stability of R proteins such as Rx and N [25,38]. Besides functioning as co-chaperone, Sgt1 also acts downstream of R proteins as silencing of *sgt1* suppressed HR induced by overexpression of the Rx NB domain or the extended TIR domain of RPS4 without affecting their accumulation [13^{••},27[•]]. Sgt1 has originally been identified as a member of the SCF E3 ubiquitin ligase complex that targets proteins for proteolysis by the proteasome [39]. Sgt1 connects the Hsp90 chaperone system to the substrate-specific arm of SCF complexes, allowing ubiquitination of Hsp90 client proteins [40]. Hence, besides its role in regulating the stability of R proteins, or their downstream partners, Sgt1 has been proposed to be involved in the removal of negative regulators that control resistance responses [40,41[•]]. Future studies aimed at the identification of such Sgt1 interacting regulators could aid in identifying its function(s).

Until now, two proteins have been reported to interact specifically with NB-ARC domains: the HRT interactor CRT1 (compromise recognition of turnip crinkle virus) and RIN13 (RPM1 interacting protein 13) [42]. CRT1 is a member of the GHKL (gyrase, Hsp90, histidine kinase, MutL) ATPase/kinases superfamily and is distantly related to the chaperone Hsp90 [43[•]]. Mutation/silencing of CRT1 confers TCV susceptibility in conjunction with an altered, HR-like response to the TCV elicitor. This suggests an early function of CRT1 in HRT-mediated resistance signalling in which the impaired defence response results in poor viral containment triggering trailing necrosis due to the residual HRT activity [43[•]]. CRT1 binds multiple plant NB-LRR proteins. Although it is not yet known to which subdomain(s) in NB-LRR proteins CRT1 binds, CRT1 is unlikely to be the component that provides specificity for downstream signalling components for CNLs and TNLs, as it associates with members of both classes [43].

RIN13 interacts strongly with the CC-NB domain of RPM1 and weakly to that of RPS2, whereas it does not bind the TNL RPP5 [42]. Arabidopsis lines in which RIN13 is silenced, or knocked out, showed normal HR

but impaired resistance to *Pseudomonas* strains carrying AvrRPM1, resembling the *crt1* phenotype. RIN13 is a plant-specific protein with orthologues in rice, whose precise function remains unknown [42].

Interactors of the N-terminal domain of NB-LRR proteins are very diverse and often show high specificity to their interacting partner (Figure 3). Three of them are predicted to be involved in nuclear processes and can be linked to transcriptional processes (WRKY, TIP49a) or nucleo-cytoplasmic transport (RanGAP2) (reviewed in [6]; Table 1). The other interactors encode kinases (Pto and PBS), a rhodanese sulfurtransferase (NRIP1), E3 ligases (RIN2 and RIN3) or are involved in basal defence via an unknown mechanism (e.g. Rin4 [44]) (for references see Table 1). Null mutants of these interactors impaired defence/HR signalling of the affected R protein. For Pto, RIN4, NRIP1 and PBS1, it has been shown that they act upstream of R protein activation as they are directly targeted by the AVR proteins. Different models have been proposed that explain the function of these N-terminal interactors. One view is that they could encode virulence targets (guard model, [8]), but it has also been suggested that they could represent target-mimics (decoy model, [9]). In both models, the N-terminal interactors assist the R protein with recognition of the pathogen, which fits the model proposed in Figure 2. The subcellular localization of the interactor in this model indicates the site of action for the effector, rather than that for the R protein. NB-LRR R proteins that directly interact with their cognate effector protein apparently do not need an N-terminal adaptor for activation. Future studies focussing on N-terminal interactors (RIN2, RIN3, WRKY and RanGAP2) for which no AVR binding has (yet) been found could clarify whether the CC/TIR domain indeed aids perception rather than downstream signalling.

Taken together, proteins interacting with the LRR domain in general seem to be involved in stabilizing R protein complexes. Inherent instability of these proteins may explain the difficulties in producing these proteins in heterologous expression systems and their tendency to form aggregates herein [45,46]. At least a subset of N-terminal interactors also interacts with the cognate effector protein, which supports the involvement of this domain in pathogen recognition (Figure 2). The subcellular localization of the interactors could provide clues to the actual virulence function of the effectors.

Conclusions and future prospects

Exploration of intra- and intermolecular interactions of NB-LRR R proteins has significantly improved our understanding of their activation during defence signalling. Unfortunately, our knowledge is still fragmentary as only for a limited number of NB-LRR proteins intramolecular interaction data are available and interactors of only a handful of R proteins have been identified. Although the

overall similarity in structure of different NB-LRR proteins suggests conserved molecular functions for the specific domains, it is already clear from the few cases analyzed that there are differences in the exact mechanisms by which they perceive pathogens and activate host defences.

In the model proposed here the major role for the highly variable N-terminal domain lies in pathogen recognition rather than signalling. Uncovering the function of the interactors of this domain is required to validate this model. Of specific interest will be the identification of proteins interacting with the conserved NB-ARC domain, as this seems to be the main integrator converting pathogen recognition into defence activation. These proteins may be different for TNL and CNL proteins. Genetic screens aimed at their identification have so far been unsuccessful in unveiling their identity, perhaps due to lethality or redundancy. Possibly, targeted proteomics approaches using NB-LRR proteins as baits will turn out to be a more fruitful approach. Another major challenge for the future will be the elucidation of the 3D structure of R proteins, preferably in the different conformational states, as this will be key to fully elucidate the molecular mechanism underlying their function.

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This paper builds on the work presented in [11] where it was shown that a decomposed NB-LRR protein can still function when its separated domains are co-expressed *in planta*. Based on domain swaps with Rx and Gpa2 and subsequent Co-IPs the authors could map recognition specificity to the C-terminal part of the LRR and the ARC1 subdomain was shown to be responsible for LRR binding. Inappropriate pairings of LRR and ARC2 resulted in autoactivation, suggesting that interplay between the LRR and ARC2 domains confers autoinhibition.
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