

EMBO Member's Review

Rumble in the nuclear jungle: compartmentalization, trafficking, and nuclear action of plant immune receptors

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Plants and animals have evolved structurally related innate immune sensors inside cells to detect the presence of microbial molecules. An evolutionary ancient folding machinery becomes engaged for the synthesis of autorepressed receptor forms in both kingdoms. The receptors act as regulatory signal transduction switches and are activated upon direct or indirect perception of non-self structures. Recent findings indicate that nucleo-cytoplasmic partitioning and nuclear activity is critical for the function of several plant immune sensors, thereby linking receptor function to transcriptional reprogramming of host cells for pathogen defense. This implies short signalling pathways and reveals parallels with regulatory control mechanisms of animal steroid receptors.

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Introduction

Plants have evolved two classes of immune receptors to detect non-self molecules. One class consists of membrane-resident pattern recognition receptors (PRRs) that detect microbe-associated molecular patterns (MAMPs). MAMPs are evolutionarily conserved structures that include components of fungal cell walls such as chitin (*N*-acetyl-chitooligosaccharide oligomers), most likely lipopolysaccharides (LPS) from gram-negative bacteria, as well as short peptides derived from bacterial flagellin or the elongation factor EF-Tu (Zipfel and Felix, 2005). Few MAMP receptors have been isolated to date, but include the *Arabidopsis* plasma membrane-resident receptor-like kinases FLS2 and EFR, recognizing flagellin and EF-TU-derived peptides flg22 and elf18, respectively (Gomez-Gomez and Boller, 2000; Zipfel *et al*,

2006). In rice, the plasma membrane-anchored CEBiP chitin receptor contains two extracellular LysM domains, a module implicated in peptidoglycan-binding, but lacks an intracellular kinase domain (Bateman and Bycroft, 2000; Kaku *et al*, 2006). MAMP-triggered signalling pathways leading to termination of microbial pathogenesis are genetically poorly defined. However, biochemical evidence points to close links between MAMP-triggered immune responses and changes of ion fluxes across and production of reactive oxygen species (ROS) on the outer surface of the plasma membrane within minutes of MAMP perception, induction of mitogen-activated protein (MAP) kinase signalling, as well as transcriptional activation of early defense-response genes. During interactions with virulent pathogens, the PRR-triggered defense system confers only weak immune responses that allow moderate pathogen growth (Chisholm *et al*, 2006). This immune weakening is mediated by pathogen-delivered effectors and involves the direct or indirect suppression of MAMP-triggered signalling (Fujikawa *et al*, 2006; He *et al*, 2006; Melotto *et al*, 2006; Nomura *et al*, 2006; de Torres-Zabala *et al*, 2007).

Plant resistance (R) proteins define a second mainly intracellular immune receptor class that have the capacity to detect directly or indirectly isolate-specific pathogen effectors, encoded by avirulence (*AVR*) genes. Like PRR-triggered immune responses, R protein-conditioned immunity is also linked to ROS accumulation and to defense gene activation, but differs both quantitatively and kinetically from the former, typically leading to host cell death at attempted invasion sites (Shirasu and Schulze-Lefert, 2000; Tao *et al*, 2003; Caldo *et al*, 2004). This 'hypersensitive response' is thought to limit the spread of infection. Because PRR- and R protein-triggered output responses are similar, it is possible that the signalling pathways converge.

Rather than repeating recent reviews on effector perception mechanisms by R proteins, we focus on recent insights in post-recognition R protein signalling, receptor compartmentalization and dynamics, as well as emerging links to the transcriptional machinery. In vertebrates, microbial molecules are detected inside cells by a class of sensors of the innate immune system known as NOD-leucine-rich repeats (NOD-LRR), NOD-like receptors (NLRs), NACHT-LRR, or CATERPILLER proteins that are structurally related to plant R proteins. We discuss the engagement of evolutionarily conserved proteins for immune receptor function in both kingdoms in the context of shared receptor folding/stabilization mechanisms. Finally, we compare emerging regulatory features of intracellular immune receptor function with steroid receptor regulation in vertebrates.

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Intracellular immune sensors act as signal transduction switches

A central nucleotide binding (NB) domain and C-terminal LRRs are common structural modules found in plant R and vertebrate NLRs (Figure 1). In contrast, a structurally diverse range of domains was apparently co-opted during evolution N-terminal to the NB domain, including coiled coil (CC) or TOLL/interleukin-1 receptor (TIR) domains in plants and in vertebrates a caspase recruitment domain (CARD), or pyrin domain (PYD), or baculovirus IAP repeats (BIRs). The central NB domain is part of a larger domain, called NB-ARC, due to its occurrence in plant R proteins, the apoptotic regulator human apoptotic protease-activating factor 1 (APAF-1), and its *Caenorhabditis elegans* homolog CED-4 (van der Biezen and Jones, 1998). NB-ARC domain-containing proteins belong to the family of STAND (signal transduction ATPases with numerous domains) NTPases that are found in archaea, bacteria, fungi, plants, and animals (Leipe *et al.*, 2004). STAND ATPases are modular proteins and display a wide range of fusions to domains involved in protein-protein or protein-DNA interactions, small-molecule-binding domains, as well as catalytic domains involved in signal transduction (Leipe *et al.*, 2004). These proteins are considered to act as regulatory signal transduction switches. A critical aspect of this switching is reversible, NTP hydrolysis-powered, conformational changes that are relayed to effector domains. STAND NTPases are unusual, because the regulatory switch, scaffolding, and occasionally, sensory as well as signal-generating moieties are integrated into a single multidomain protein (Leipe *et al.*, 2004).

The crystal structures of APAF-1 and CED-4 revealed four NB-ARC subdomains, the nucleotide-binding site (NBS) plus

three ARC subdomains (ARC1-ARC3) (Riedl *et al.*, 2005; Yan *et al.*, 2005) (note that ARC3 is absent in R proteins and substituted by a short linker of yet unknown structure). Importantly, the nucleotide in APAF-1 and CED-4 is bound at the interface of NBS, ARC1, and ARC2 subdomains and, depending on the presence of ATP or ADP, brings about markedly different conformers. A highly conserved MHD-motif (hxhHD) of plant R proteins is located in the ARC2 subdomain (Takken *et al.*, 2006). The histidine next to the aspartate in this motif directly interacts with the β -phosphate in APAF-1 (Riedl *et al.*, 2005). Mutagenesis of either the histidine or aspartate in several R proteins as well as human NOD2 results in autoactivation (Bendahmane *et al.*, 2002; Shirano *et al.*, 2002; Tanabe *et al.*, 2004; Howles *et al.*, 2005; Tameling *et al.*, 2006), indicating that these residues are important to keep the receptors in an inactive form. Biochemical analysis of two autoactivating mutations of the tomato I-2 R protein, which confers resistance to the fungal pathogen *Fusarium oxysporum*, showed *in vitro* markedly reduced ATP hydrolysis but did not affect nucleotide binding (Tameling *et al.*, 2006). This suggests that the ATP bound form is the 'on state' whilst ATP hydrolysis switches the protein back to the 'off state' (Figure 2A).

Autoactivating mutations in plant R proteins and human NOD2 also map in the linker region between ARC2 and the LRR region (Zhang *et al.*, 2003), as well as in the N-terminal part of the LRRs (Bendahmane *et al.*, 2002; Shirano *et al.*, 2002; Tanabe *et al.*, 2004; Takken *et al.*, 2006). This points to the existence of additional receptor regions that keep the protein in an autorepressed form in the absence of a cognate pathogen effector. Indeed, domain swap experiments between the highly sequence-related potato CC-NB-LRR-type Rx and GPA2 R proteins produced autoactive variants upon

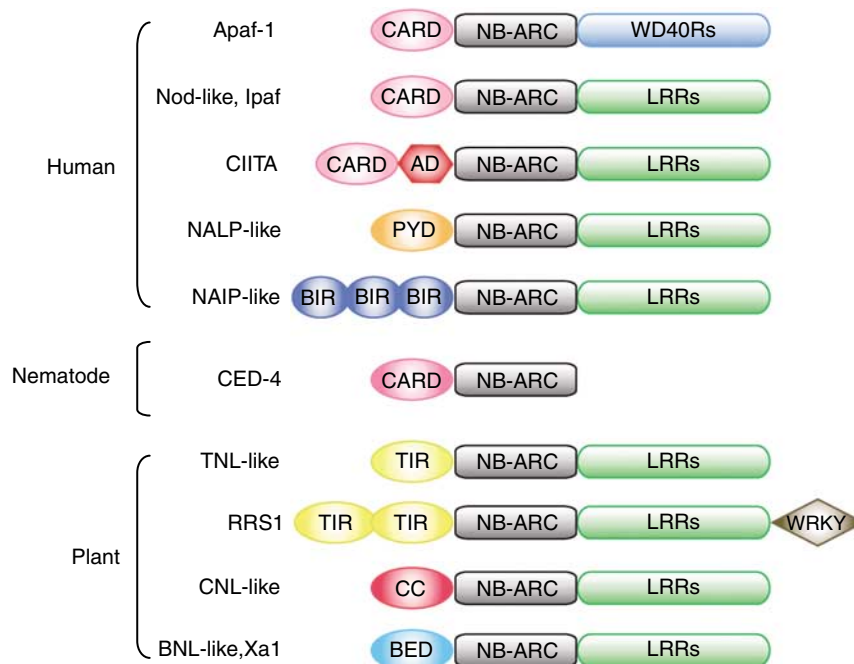


Figure 1 Intracellular immune sensors of microbial structures in plants and animals. Graphic representation of the tripartite modular structure of human NLRs, the apoptotic regulators human Apaf-1 and *C. elegans* CED-4, and R proteins. NB and ATPase activity is mediated by the central NB-ARC domain. The C-terminal LRRs are believed to sense, directly or indirectly, microbe-derived ligands. Structurally diverse N-terminal effector domains include the CARD, PYR, BIR, TIR, CC, and BEAF and BED. A WRKY DNA-binding domain is located at the C-terminus of RRS1. AD, activation domain.

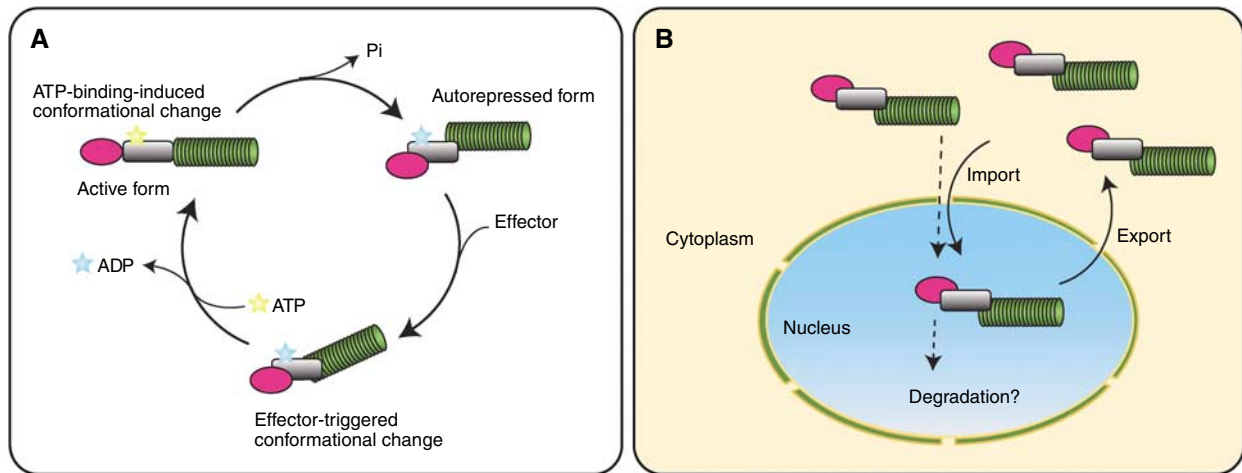


Figure 2 Intracellular immune sensors act as a regulatory signal transduction switch and are translocated into the nucleus. **(A)** In the absence of cognate microbial structures, the immune sensor is in an autorepressed, ADP-bound resting state. Direct or indirect perception of a microbial effector is thought to induce first a conformational change in the NB-ARC domain allowing exchange of ADP by ATP. This is believed to trigger a second conformational change in the N-terminal effector domain, thereby activating the receptor. The ATPase activity of the NB-ARC domain switches the conformation of the protein back to its resting state. **(B)** Translocation of immune receptors into the nucleus could either involve continuous cycling or is unidirectional. In the latter scenario, a presumed nuclear degradation pathway might explain disproportionately low levels of nuclear receptor pools.

inappropriate pairings of ARC2 and LRR domains (Rairdan and Moffett, 2006), suggesting that intramolecular interactions between these two domains regulate the receptor's transition from an autorepressed to an active state. The importance of intramolecular domain-domain interactions in Rx is also illustrated by the reconstitution of effector-dependent Rx activity following *in planta* coexpression of nonoverlapping receptor fragments (Moffett *et al.*, 2002). Together, this has led to a model in which the direct or indirect recognition of pathogen effectors by the polymorphic LRR region initiates a first conformational change (Figure 2A). This facilitates exchange of ADP by ATP, which in turn is thought to trigger a second conformational change that renders the respective N-terminal effector domain (CC, TIR, CARD, PYR, BIR) accessible for associations with downstream targets. Subsequent ATP hydrolysis switches the receptor back to its autorepressed form (Takken *et al.*, 2006).

Nuclear action of R proteins

How plant NB-LRR proteins activate immune responses following recognition of pathogen-derived effectors has been a major question since the molecular isolation of the founding members of this protein family (Bent *et al.*, 1994; Whitham *et al.*, 1994). Recent findings suggest that members of the CC- and TIR-type receptor families function in the nucleus. Allelic barley MLA CC-type receptors recognize isolate-specific effectors of the grass powdery mildew fungus, *Blumeria graminis* f sp *hordei* (Ridout *et al.*, 2006). Fractionation of cell extracts using transgenic plants that express native levels of epitope-tagged MLA as well as visualization of a fluorochrome-marked MLA in living epidermal cells localized the majority of the receptor to the soluble cytoplasmic fraction and approximately 5% to the nucleus (Bieri *et al.*, 2004; Shen *et al.*, 2007). Perturbation of nucleocytoplasmic MLA10 partitioning by expression of a receptor fusion protein containing a nuclear export signal

(NES), which enhances nuclear export over import, abrogated MLA10-specified disease resistance (Shen *et al.*, 2007). Similarly, adding a NES to the tobacco TIR-type N receptor, which conditions immunity against the tobacco mosaic virus (TMV) upon recognition of the p50 TMV replicase, impaired both N nuclear accumulation and TMV disease resistance (Whitham *et al.*, 1994; Burch-Smith *et al.*, 2007). Nuclear action of MLA and N was unexpected, because both proteins lack a canonical nuclear localization signal (NLS). Unlike this, the *Arabidopsis* TIR-type RPS4 protein, conditioning immunity to *Pseudomonas syringae* strains expressing avrRps4 (Gassmann *et al.*, 1999), contains a bipartite NLS, and this targeting signal is required for both nuclear import and disease resistance (Wirthmueller *et al.*, 2007). Similar to barley MLA, less than 10% of total cellular RPS4 was found in *Arabidopsis* nuclei preparations, whereas the bulk of the receptor associates with endosomes. Re-inspection of all 71 annotated *Arabidopsis* TNL and 54 CNL subfamily members (Meyers *et al.*, 2003) reveals a widespread potential for nuclear localization of other R proteins; using the WoLF PSORT subcellular localization prediction (<http://wolfpsort.org/>), 51 TNL and 39 CNL protein models contain predicted monopartite or bipartite NLSs. Given the fact that in yeast 43% of known nuclear proteins enter the nucleus without discernible NLSs (Lange *et al.*, 2007), the utilization of NLS-dependent and seemingly NLS-independent nuclear import pathways for plant R proteins is not surprising.

Transcriptional reprogramming of plant cells upon pathogen attack is extensive, affecting between 3 and 12% of the 24 000 tested *Arabidopsis* genes upon fungal or bacterial challenge, respectively (Nishimura *et al.*, 2003; Thilmony *et al.*, 2006). How the perception of non-self structures by PRRs and R proteins leads to transcriptional activation of defense-response genes has been a long-standing question. In this context, nuclear activities of barley MLA, tobacco N, and *Arabidopsis* RPS4 reveal novel insight. Quantitative fluorescence lifetime imaging of fluorochrome-tagged receptor was employed to visualize *in vivo* in nuclei an effector-dependent

physical association between the MLA10 receptor and two WRKY transcription factors (*HvWRKY1* and *HvWRKY2* TFs; (Shen *et al.*, 2007), suggesting that the TFs serve as immediate downstream targets of the activated receptor. This protein-protein association is mediated by the invariant N-terminal CC domain of allelic MLA receptors. Because the polymorphic C-terminal LRR region of MLA has been shown to determine recognition specificity (Shen *et al.*, 2003), it is possible that this region senses, directly or indirectly, the presence of powdery mildew effectors, while the N-terminal CC of the activated receptor acts as a signal relay moiety to the WRKY TFs. Accordingly, different structural modules at opposite ends of the receptor might account for sensory and signal transmission subfunctions. Whilst it remains to be seen whether MLA and RPS4 proteins detect the corresponding effectors in the cytoplasm and/or nucleus, the cytoplasmic pool of tobacco N appears to detect the TMV p50 viral effector. When the p50 effector was fused to the NES, thereby depleting the nuclear p50 pool and enforcing cytoplasmic localization, plant cells retained the ability to trigger N-mediated disease resistance (Burch-Smith *et al.*, 2007). Thus, sensory and signal transmission activities of N might take effect in different compartments.

Tobacco N interacts with two squamosa promoter-like (SPL) TFs that are required for TMV disease resistance (D Kumar, personal communication). As viral effector recog-

nition by N occurs in the cytoplasm, the N interacting SPLTFs could serve as targets of the activated receptor. The SPL gene family represents a group of structurally diverse transcription factors found apparently only in plants (Cardon *et al.*, 1999). Notably, loss-of-function mutations in the *Arabidopsis SPL14* gene render mutant plants insensitive to the mycotoxin fumonisin B1, which elicits an apoptotic form of cell death in wild-type plants as well as tissue-cultured plant and animal cells (Gilchrist, 1997; Stone *et al.*, 2005). This raises the question whether fumonisin B1- and N receptor-triggered cell death processes are mechanistically related and involve modifications of SPL TF activities. Likewise, the effector-dependent association between barley MLA10 and WRKY TFs appears to contribute to receptor-triggered disease resistance and host cell death at attempted fungal infection sites (Shen *et al.*, 2007). However, the WRKY TFs interacting with MLA act as repressors of MAMP-triggered immune responses and might have a role in preventing 'chronic inflammatory responses' and/or to dampen immune responses below a threshold that is detrimental to attacked plant cells (Xu *et al.*, 2006; Figure 3A). We hypothesized that MLA receptors may interfere with the WRKY repressor function, thereby derepressing MAMP-triggered immune responses. The derepression could amplify MAMP-triggered immune responses and, in principle, would be sufficient to drive plant cells into suicide. Thus, the effector-triggered MLA WRKY association

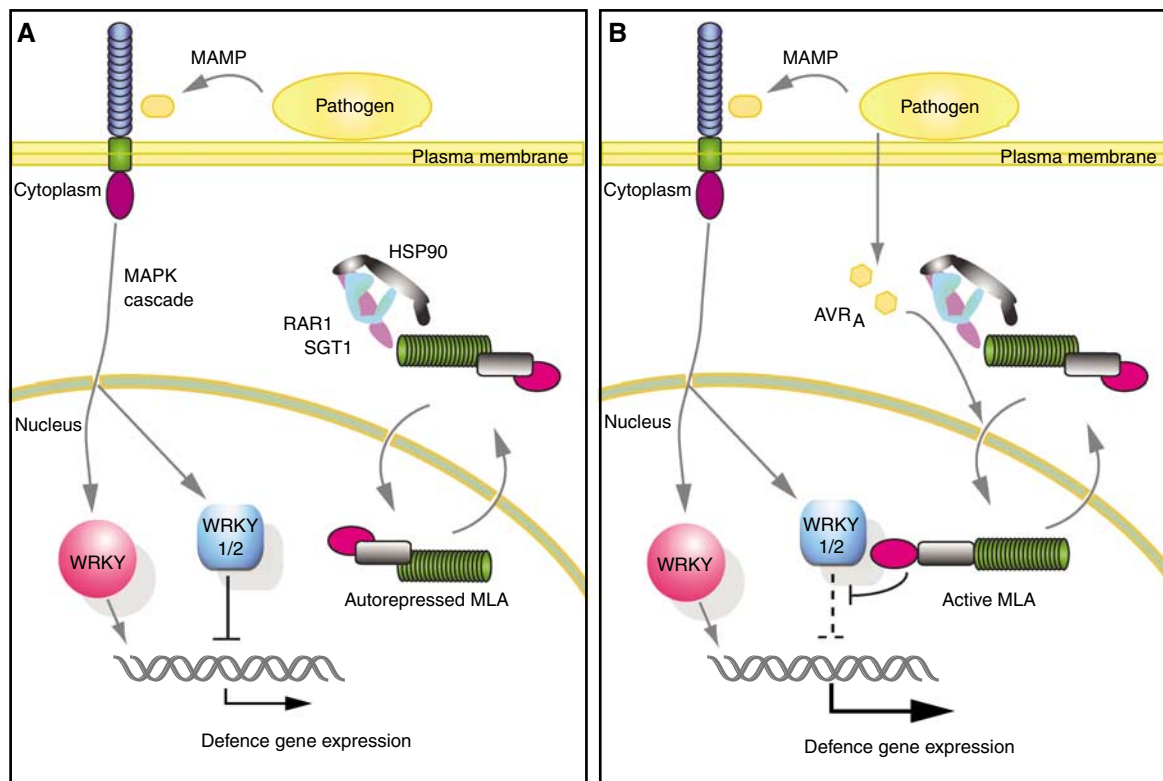


Figure 3 Nuclear action of MLA links effector-specific and MAMP-triggered immune responses. (A) One or several MAMP receptors initiate PAMP signalling via intracellular MAPK cascades, which in turn stimulate the induction of unknown WRKY transcriptional activators (pink color) and WRKY1/2 repressors (blue color). The WRKY repressors are thought to prevent chronic defense gene activation. Autorepressed MLA receptors are folded by RAR1, SGT1, and cytosolic HSP90 and might continuously cycle between nucleus and cytoplasm. (B) Integrated PAMP- and MLA-triggered immune response upon coactivation of one or several MAMP receptors and MLA by cognate powdery mildew effectors (designated AVR_A). Activated MLA stimulates nuclear association with WRKY1/2 repressors, thereby derepressing MAMP-triggered immunity. Derepression of basal defense responses is thought to amplify expression of defense-related genes (bold arrow) and might drive attacked host cells into cellular suicide. Whether AVR_A is directly or indirectly recognized by the cytoplasmic and/or nuclear MLA pool remains unknown.

could serve as nexus to integrate signals generated by PRRs and R proteins (Figure 3B). A similar regulatory logic might help to explain previous *in planta* experiments with autoactive forms of the flax TIR-NB-LRR protein L6 (Howles *et al*, 2005). Wild-type L6 confers typical race-specific immunity associated with localized cell death to strains of the flax rust fungus that carry the cognate avirulence gene, designated *AvrL567*. In recovered transgenic plants expressing autoactive L6 defense-related gene expression is chronically activated without signs of cell death. However, when the transgenic plants were challenged with flax rust isolates that are virulent on wild-type L6 plants, effective immunity was observed that was accompanied by an L6-like cell death response. Thus, while autoactive L6 alone is unable to drive plant cells into suicide, MAMPs released during fungal attack might trigger cell death-associated immune responses because of the simultaneous presence of autoactive L6.

Unlike direct links between MLA or N receptor function and the transcriptional machinery, nuclear RPS4 activity requires EDS1, a protein of unknown biochemical function(s) that lacks known chromatin- or DNA-binding domains and resides in both cytoplasmic and nuclear compartments (Feys *et al*, 2005; Wirthmueller *et al*, 2007). RPS4-triggered immunity, but not nucleo-cytoplasmic partitioning or receptor stability, is abolished in an *eds1* null mutant background. Together with an almost complete breakdown of RPS4/EDS1-dependent activation/repression of approximately 130 defense-related genes in *eds1* plants (Bartsch *et al*, 2006; Wirthmueller *et al*, 2007), this suggests that EDS1 acts as intermediary positive signal transducer between the receptor and defense gene expression.

Further evidence for transcription machinery-associated functions of plant immune sensors comes from a functional analysis of *Arabidopsis* *RRS1*, which conditions disease resistance to the bacterial pathogen *Ralstonia solanacearum* expressing the cognate effector PopP2 (Deslandes *et al*, 2003). *RRS1* is unusual because it encodes a TIR-NB-LRR R protein with a C-terminal WRKY domain (Figure 1). The latter is shared by all WRKY transcription factor family members and is known to bind to *cis*-active DNA elements, termed W-boxes (Ulker and Somssich, 2004; Yamasaki *et al*, 2005). The type III effector PopP2 carries a bipartite nuclear localization signal and is specifically targeted to host cell nuclei. Transient gene expression experiments in *Arabidopsis* protoplasts using fluorochrome-tagged *RRS1* and PopP2 demonstrated that nuclear visualization of *RRS1* requires co-expression of PopP2, while expression of *RRS1*-GFP alone did not produce a fluorescence signal (Deslandes *et al*, 2003). As *RRS1* and PopP2 were also shown to interact in yeast two-hybrid experiments, it is possible that the association with PopP2 either induces conformational changes in *RRS1*-GFP, thereby producing a detectable fluorescence signal, or that *RRS1*-GFP forms a heterocomplex, which is resistant to degradation. A 3 bp insertion mutation in *RRS1* (synonym *SHL1*) that results in the addition of a single amino acid in the WRKY domain, thereby impairing its DNA-binding activity, leads to chronic expression of defense genes and occasional cell death in the absence of the parasite (Noutoshi *et al*, 2005). One interpretation is that in healthy plants, the wild-type protein must bind to DNA to repress plant defense gene expression. In this scenario, the association between *RRS1* and PopP2 could serve as a trigger to sequester the R protein

away from DNA, thereby allowing defense gene expression. Note that this is conceptually similar to the proposed derepression of MAMP-triggered immune responses following effector-induced associations between barley MLA10 and WRKY1 or WRKY2 repressors (Shen *et al*, 2007). Similarly, the plasma membrane-tethered *Arabidopsis* RPM1 CC-NB-LRR protein, which recognizes the *P. syringae* effector AvrRpm1, can interact with a DNA polymerase II accessory protein, TIP49a, that acts as an inhibitor of plant immune responses (Holt *et al*, 2002). Similar to the results obtained for barley MLA, full-length RPM1 does not interact with TIP49a in yeast, whereas the N-terminal CC-NB part alone does. This might be explained by both full-length R proteins adopting a conformation that masks the N-terminal interacting residues in yeast. This is consistent with the idea that the association between TIP49a and RPM1 is a post-activation event. However, it remains to be shown whether RPM1 must enter the nucleus to associate with TIP49a.

The *Arabidopsis* genome contains another R gene homolog (At4g12020), in which an N-terminal WRKY DNA-binding domain is fused to a TIR-NB-LRR protein. This deduced protein contains an additional C-terminal kinase domain. Although no biological function has been assigned to the WRKY-TIR-NB-LRR-kinase to date, it is of note that the *Populus trichocarpa* genome contains 40 NB-LRR gene models, not present in *Arabidopsis*, which carry an N-terminal BEAF and DREF DNA-binding finger (BED) DNA-binding zinc-finger domain (Aravind, 2000; Tuskan *et al*, 2006). This domain is also present at the N-terminus of the rice Xa1 NB-LRR R protein to *Xanthomonas oryzae* (Figure 1; Yoshimura *et al*, 1998). Thus, it is possible that a subgroup of plant immune receptors has acquired direct DNA-binding capacity by domain co-option involving WRKY or BED domains. To date, there is only one example of nuclear activity for a human NOD-like receptor family member: the CIITA protein contains an N-terminal CARD domain and acts through direct association with DNA-binding proteins to regulate expression of all major histocompatibility class II and other genes important in antigen presentation (Figure 1; Ting *et al*, 2006). CIITA function appears to involve 'promoter loading' of MHC class II genes. Unfortunately, its potential role in sensing microbial structures remains still unclear.

Nucleo-cytoplasmic shuttling or unidirectional nuclear import?

As barley MLA, tobacco N, as well as *Arabidopsis* RPS4 each localizes to the cytoplasm and nucleus in healthy plants, nucleo-cytoplasmic partitioning is an intrinsic effector-independent feature of these receptors. This partitioning is expected to engage the nuclear import and export machinery. Indirect evidence for this comes from genetic experiments in which a gain-of-function mutation in an *Arabidopsis* TIR-NB-LRR gene was used to study the requirements of an 'autoimmunity' phenotype (Zhang *et al*, 2003; Palma *et al*, 2005; Zhang and Li, 2005). Mutant *snc1* plants express the autoactive SNC1 protein carrying a single-amino-acid substitution between the NB LRR domains, leading to chronic activation of defense responses and disease resistance to bacterial and oomycete pathogens (Zhang *et al*, 2003). Recessive mutations in two suppressor loci of the *snc1* genotype, *MOS3* and *MOS6*, each affects components required for protein passage through

the nuclear pore. MOS3 is homologous to vertebrate nucleoporin 96 (Nup96) and resides at the nuclear rim (Zhang and Li, 2005). Vertebrate Nup96 and the yeast homolog, C-Nup145p, serve as components of the conserved Nup107–160 nuclear pore subcomplex, which is localized to both sides of the nuclear pore and regulates nuclear pore complex assembly and mRNA export (Vasu and Forbes, 2001; Walther *et al.*, 2003). *MOS6* encodes importin $\alpha 3$ (Palma *et al.*, 2005), a family of proteins known to function as adapters by binding to NLS-containing cargo proteins and to importin β . The latter interacts with Nups to traverse nuclear pore complexes, thus implying mechanistically linked functions for MOS3 and MOS6 in nucleo-cytoplasmic trafficking. Functional specialization of importin family members is indicated by the fact that importin $\alpha 3$ represents one of eight importin homologs present in the *Arabidopsis* genome (Palma *et al.*, 2005). The biological significance of *snc1* suppressors comes from fully or partially restored susceptibility to virulent bacterial and oomycete pathogens in *mos3* and *mos6* plants, respectively (Palma *et al.*, 2005; Zhang and Li, 2005). One intriguing possibility is that MOS3 Nup96 and MOS6 importin $\alpha 3$ are required for nuclear import of the autoactive SNC1 protein. Alternatively, they might serve as ‘downstream’ components of a presumed nuclear SNC1 activity, for example, as gatekeepers for mRNA export of SNC1 target genes.

A physical association between the CC-NB-LRR-type Rx R protein, conditioning immunity to the PVX virus, and *Nicotiana benthamiana* Ran GTPase-activating protein 2 (*NbRanGAP2*) directly links R protein function to nucleo-cytoplasmic trafficking (Tameling and Baulcombe, 2007). Affinity purification of epitope-tagged Rx coupled to protein mass spectrometry revealed an association with *NbRanGAP2*, but not with the closely related *NbRanGAP1*. Importantly, gene silencing of *NbRanGAP2* partially compromises Rx-dependent viral disease resistance. RanGAP proteins are conserved in eukaryotes and are known to regulate the activity of the small GTPase Ran (Ras-related nuclear protein), which in turn is pivotal for trafficking of macromolecules through nuclear pores (Meier, 2007). The above-mentioned importin $\alpha \beta$ complex, loaded with NLS-containing cargo, dissociates in the nucleus upon binding of RanGTP, thereby permitting cytoplasmic reshuttling of importin α and β (note that RanGDP localizes to the cytoplasmic side of the nuclear envelope; (Merkle, 2001; Xu and Massague, 2004; Meier, 2007). Vertebrate RanGAP and plant RanGAP contain kingdom-specific domains, but appear to be both anchored to the outer surface of the nuclear envelope through different nuclear envelope-associated proteins (Rose and Meier, 2001). Like barley MLA and tobacco N, Rx lacks an obvious NLS and localizes to the cytoplasm and nucleus (J Bakker, personal communication). Thus, it is conceivable that *NbRanGAP2* plays a direct role in the nuclear passage of Rx: by carrying Rx into the nucleus or by mediating loading of Rx to other NLS-containing carriers. If this were true, then gene silencing of *NbRanGAP2* is expected to deplete the nuclear Rx pool. Alternatively, Rx could form a preformed recognition complex with *NbRanGAP2* in healthy plants. In this scenario, the PVX coat protein, which is recognized by Rx (Farnham and Baulcombe, 2006), might target *NbRanGAP2* for viral dissemination. Viral manipulation of *NbRanGAP2* would then be indirectly sensed by Rx.

If the above discussed immune receptors cycle continuously between nucleus and cytoplasm, then differential nuclear import and export rates or a cytoplasmic retention mechanism could account for the observed ~ 16 -fold excess of cytoplasmic barley MLA and *Arabidopsis* RPS4 receptor pools (Shen *et al.*, 2007; Wirthmueller *et al.*, 2007). Alternatively, if nuclear import is unidirectional, then low levels of the nuclear receptor could be the consequence of a specific nuclear degradation mechanism. As the cycle of ATP/ADP binding and ATP hydrolysis in the NBS of R proteins is likely operating at a low level in the absence of cognate effectors (Figure 2A; Takken *et al.*, 2006), thereby running the risk of autoactivation, a presumptive nuclear receptor degradation mechanism could serve as additional safeguard to prevent inappropriate signalling and/or might have a role in signal desensitization (Figure 2B and below).

Folding, stabilization, and degradation of plant and animal immune sensors

Genetic and biochemical experiments revealed evolutionarily conserved proteins in vertebrates and plants that appear to serve critical functions in maintaining ‘optimal’ preactivation receptor levels, possibly by coupling receptor folding and degradation pathways. The co-chaperone-like proteins RAR1 and SGT1, as well as the cytosolic HSP90 chaperone were originally identified in plants by mutational screens as essential components of a subset of R protein-triggered immune responses to diverse plant pathogens (Shirasu and Schulze-Lefert, 2003). Loss of disease-resistance function in *rar1* or *sgt1* or *hsp90* mutant plants typically results in a severe depletion of R protein levels in the absence of parasite (Tornero *et al.*, 2002; Hubert *et al.*, 2003; Lu *et al.*, 2003; Belkhadir *et al.*, 2004; Bieri *et al.*, 2004; Holt *et al.*, 2005; Azevedo *et al.*, 2006). Specific mutations in *HSP90*, inactivating its intrinsic ATPase activity, and direct physical associations between the chaperone and R proteins suggest that the latter are HSP90 ‘clients’ and become stabilized by the chaperone (Figure 3A and B; Hubert *et al.*, 2003). As SGT1 and (metazoan) RAR1 share structural similarities with co-chaperones and bind to each other as well as to HSP90 (Azevedo *et al.*, 2002; Shirasu and Schulze-Lefert, 2003; Takahashi *et al.*, 2003; Bieri *et al.*, 2004; Liu *et al.*, 2004), the former two are thought to act as co-chaperones, possibly by positively modulating HSP90 activity on its R protein clients. A link to protein degradation comes from the finding that RAR1 and SGT1 each interact with subunits of the COP9 signalosome, a multiprotein complex of the ubiquitin-proteasome pathway (Serino and Deng, 2003), and from an association of SGT1 with SCF ubiquitin ligase components (Azevedo *et al.*, 2002; Liu *et al.*, 2002). The receptors themselves could serve as degradation targets. As R protein levels are typically decreased in recessive *rar1* and *sgt1* single mutants, the corresponding wild-type genes could antagonize a default receptor degradation pathway such that receptor folding and degradation processes are coupled. A seemingly antagonistic control of R protein stability by RAR1 and SGT1, as inferred from a reduction and partial recovery of R protein levels in *Arabidopsis rar1* single and *rar1 sgt1b* double mutants, respectively, could alternatively be explained by a compensatory SGT1b-like activity supplied by the closely related *SGT1a* homolog (Holt *et al.*, 2005; Azevedo *et al.*, 2006).

Repressors of immune responses/cell death that associate with the receptors before or after effector recognition, serve as additional candidate targets of the COP9/SCF ubiquitin degradation machinery (Azevedo *et al*, 2002; Liu *et al*, 2002). Thus, it will be interesting to examine the fate of barley WRKY1/2 repressors or *Arabidopsis* TIP49a after receptor activation.

Recent findings show that human homologs of SGT1 and cytosolic HSP90 form complexes with the CARD domain-containing NOD1 and NOD2 immune sensors as well as with several other NLRs including NALP3 (Hahn, 2005; da Silva Correia *et al*, 2007; Mayor *et al*, 2007). The latter contains an N-terminal PYR instead of a CARD domain. Application of geldanamycin, an HSP90 inhibitor, or depletion of SGT1 by small interfering RNA from cultured cells revealed a requirement for bacterial peptidoglycan-triggered NOD1 and NOD2 immune sensor function. Substantial decreases of NOD1 or NALP3 levels in geldanamycin-treated cells that could be antagonized by lactacystin, a proteasome inhibitor, suggests that shared biochemical mechanisms contribute to the folding/stability of signalling-competent immune sensors in animals and plants (Hahn, 2005; Mayor *et al*, 2007). Functional dependence on SGT1 and HSP90 of receptors carrying unrelated N-terminal domains (TIR, CC, CARD, PYR) as well as direct binding of SGT1-HSP90 complexes to their LRRs indicate that folding of the signalling-competent form occurs primarily via the polymorphic C-terminal receptor region. Despite these advances in assigning RAR1, SGT1, and HSP90 a function in folding/stability of preactivated immune sensors, it remains possible that they fulfill additional roles in post-activation signalling.

Both controlled folding and nucleo-cytoplasmic trafficking of intracellular immune receptors are regulatory features that are strikingly reminiscent of animal steroid receptor regulation (Pratt and Toft, 2003; Pemberton and Paschal, 2005). A cytoplasmic hetero-complex consisting of several heat shock proteins—including HSP90, HSP70, and HSP40—as well as co-chaperones forces an opening of the steroid-binding cleft, driven by heat-shock protein ATPase activity, such that the binding pocket can be accessed by a steroid ligand. Activation occurs in a stepwise manner, driven by ATP hydrolysis, to produce first a ‘primed complex’ and then a steroid-binding competent complex. Translocation of cytoplasmic steroid hormone receptor complexes to the nucleus upon binding of cognate steroids and docking to hormone response DNA elements is a hallmark of this receptor family (McKenna and

O’Malley, 2002). Recent data suggest that steroid-binding may regulate a chaperone-dependent step that occurs after recognition of the NLS in these receptors (Davies *et al*, 2002; Freedman and Yamamoto, 2004). Nucleocytoplasmic shuttling of steroid receptors also provides a nexus for crosstalk with kinase stress pathways (Shank and Paschal, 2005). For example, epidermal growth factor signalling through a MAP kinase pathway leads to phosphorylation of the progesterone receptor, MAP kinase-dependent nuclear export, and subsequent degradation in the cytoplasm (Qiu *et al*, 2003).

Conclusion

It remains to be seen how many plant NB-LRR proteins function in the nucleus. The widespread occurrence of NLSs in *Arabidopsis* TIR- and CC-type receptor subfamilies is an indication that their nuclear location might not be an exception. Direct targeting of the transcriptional machinery by NB-LRR proteins as in the case of MLA receptors implies a short signalling pathway that may not depend on authentic signalling components. This could explain why mutational approaches in plants have failed so far in identifying signalling mutants that exclusively compromise NB-LRR receptor function. Given the functional and structural similarities of plant R and vertebrate NLR immune sensors, it will not be surprising if nuclear pools exist for NLR family members besides CIITA. Derepression of MAMP-triggered immune responses through MLA receptor interference with WRKY repressors is likely to be only one of several potential convergence points between MAMP- and R protein-triggered signalling pathways. Convergence points could also be generated by MAMP-triggered and MAP kinase-dependent R protein phosphorylation, in turn modulating effector-triggered receptor activity and/or nucleo-cytoplasmic receptor partitioning. In this context, nuclear translocation of a plant MAP kinase upon treatment of cell cultures with an oomycete-derived MAMP deserves special note (Ligterink *et al*, 1997). If nuclear action of R proteins is a widespread phenomenon, one would expect that evolution favored diverse interception points with the transcriptional machinery to avoid an Achilles’ heel for immune sabotage by pathogens. Thus, whether different nuclear immune sensors target the same, different, or overlapping chromatin sites and how this translates into spatio-temporal changes of defense gene expression patterns could become a focus of future experimentation.

References

- Aravind L (2000) The BED finger, a novel DNA-binding domain in chromatin-boundary-element-binding proteins and transposases. *Trends Biochem Sci* **25**: 421–423
- Azevedo C, Betsuyaku S, Peart J, Takahashi A, Noel L, Sadanandom A, Casais C, Parker J, Shirasu K (2006) Role of SGT1 in resistance protein accumulation in plant immunity. *EMBO J* **25**: 2007–2016
- Azevedo C, Sadanandom A, Kitagawa K, Freialdenhoven A, Shirasu K, Schulze-Lefert P (2002) The RAR1 interactor SGT1, an essential component of R Gene-triggered disease resistance. *Science* **295**: 2073–2076
- Bartsch M, Gobbato E, Bednarek P, Debey S, Schultze JL, Bautor J, Parker JE (2006) Salicylic acid-independent ENHANCED DISEASE SUSCEPTIBILITY1 signaling in *Arabidopsis* immunity and cell death is regulated by the monooxygenase FMO1 and the nudix hydrolase NUDT7. *Plant Cell* **18**: 1038–1051
- Bateman A, Bycroft M (2000) The structure of a LysM domain from *E coli* membrane-bound lytic murein transglycosylase D (MltD). *J Mol Biol* **299**: 1113–1119
- Belkhadir Y, Subramaniam R, Dangl JL (2004) Plant disease resistance protein signaling: NBS-LRR proteins and their partners. *Curr Opin Plant Biol* **7**: 391–399
- Bendahmane A, Farnham G, Moffett P, Baulcombe D (2002) Constitutive gain-of-function mutants in a nucleotide binding site-leucine rich repeat protein encoded at the Rx locus of potato. *Plant J* **32**: 195–204
- Bent AF, Kunkel BN, Dahlbeck D, Brown KL, Schmidt R, Giraudat J, Leung J, Staskawicz BJ (1994) Rps2 of *Arabidopsis thaliana*—

- a leucine-rich repeat class of plant-disease resistance genes. *Science* **265**: 1856–1860
- Bieri S, Mauch S, Shen Q-H, Peart J, Devoto A, Casais C, Ceron F, Schulze S, Steinbiss H-H, Shirasu K, Schulze-Lefert P (2004) RAR1 positively controls steady state levels of barley MLA resistance proteins and enables sufficient MLA6 accumulation for effective resistance. *Plant Cell* **16**: 3480–3495
- Burch-Smith TM, Schiff M, Caplan JL, Tsao J, Czymbek K, Dinesh-Kumar SP (2007) A novel role for the TIR domain in association with pathogen-derived elicitors. *PLoS Biol* **5**: 501–514
- Caldo RA, Nettleton D, Wise RP (2004) Interaction-dependent gene expression in Mla-specified response to barley powdery mildew. *Plant Cell* **16**: 2514–2528
- Cardon G, Hohmann S, Klein J, Nettlesheim K, Saedler H, Huijser P (1999) Molecular characterisation of the *Arabidopsis* SBP-box genes. *Gene* **237**: 91–104
- Chisholm ST, Coaker G, Day B, Staskawicz BJ (2006) Host-microbe interactions: Shaping the evolution of the plant immune response. *Cell* **124**: 803–814
- da Silva Correia J, Miranda Y, Leonard N, Ulevitch R (2007) SGT1 is essential for Nod1 activation. *Proc Natl Acad Sci USA* **104**: 6764–6769
- Davies TH, Ning YM, Sanchez ER (2002) A new first step in activation of steroid receptors—hormone-induced switching of FKBP51 and FKBP52 immunophilins. *J Biol Chem* **277**: 4597–4600
- de Torres-Zabala M, Truman W, Bennett MH, Lafforgue G, Mansfield JW, Egea PR, Bogre L, Grant M (2007) *Pseudomonas syringae* pv. tomato hijacks the *Arabidopsis* abscisic acid signalling pathway to cause disease. *EMBO J* **26**: 1434–1443
- Deslandes L, Olivier J, Peeters N, Feng DX, Khounloham M, Boucher C, Somssich L, Genin S, Marco Y (2003) Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. *Proc Natl Acad Sci USA* **100**: 8024–8029
- Farnham G, Baulcombe DC (2006) Artificial evolution extends the spectrum of viruses that are targeted by a disease-resistance gene from potato. *Proc Natl Acad Sci USA* **103**: 18828–18833
- Feys BJ, Wiermer M, Bhat RA, Moisan LJ, Medina-Escobar N, Neu C, Cabral A, Parker JE (2005) *Arabidopsis* SENESCENCE-ASSOCIATED GENE101 stabilizes and signals within an ENHANCED DISEASE SUSCEPTIBILITY complex in plant innate immunity. *Plant Cell* **17**: 2601–2613
- Freedman ND, Yamamoto KR (2004) Importin 7 and importin alpha/importin beta are nuclear import receptors for the glucocorticoid receptor. *Mol Biol Cell* **15**: 2276–2286
- Fujikawa T, Ishihara H, Leach JE, Tsuyumu S (2006) Suppression of defense response in plants by the avrBs3/pthA gene family of *Xanthomonas* spp. *Mol Plant Microbe Interact* **19**: 342–349
- Gassmann W, Hinsch M, Staskawicz B (1999) The *Arabidopsis* RPS4 bacterial-resistance gene is a member of the TIR-NBS-LRR family of disease-resistance genes. *Plant J* **20**: 265–277
- Gilchrist DG (1997) Mycotoxins reveal connections between plants and animals in apoptosis and ceramide signaling. *Cell Death Differ* **4**: 689–698
- Gomez-Gomez L, Boller T (2000) FLS2: An LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*. *Mol Cell* **5**: 1003–1011
- Hahn JS (2005) Regulation of Nod1 by Hsp90 chaperone complex. *FEBS Lett* **579**: 4513–4519
- He P, Shan L, Lin NC, Martin GB, Kemmerling B, Nurnberger T, Sheen J (2006) Specific bacterial suppressors of MAMP signaling upstream of MAPKKK in *Arabidopsis* innate immunity. *Cell* **125**: 563–575
- Holt BF, Belkhadir Y, Dangl JL (2005) Antagonistic control of disease resistance protein stability in the plant immune system. *Science* **309**: 929–932
- Holt III BF, Boyes DC, Ellerström M, Siefers N, Wiig A, Kauffman S, Grant MR, Dangl JL (2002) An evolutionarily conserved mediator of plant disease resistance gene function is required for normal *Arabidopsis* development. *Dev Cell* **2**: 807–817
- Howles P, Lawrence G, Finnegan J, McFadden H, Ayliffe M, Dodds P, Ellis J (2005) Autoactive alleles of the flax L6 rust resistance gene induce non-race-specific rust resistance associated with the hypersensitive response. *Mol Plant Microbe Interact* **18**: 570–582
- Hubert DA, Tornero P, Belkhadir Y, Krishna P, Takahashi A, Shirasu K, Dangl JL (2003) Cytosolic HSP90 associates with and modulates the *Arabidopsis* RPM1 disease resistance protein. *EMBO J* **22**: 5679–5689
- Kaku H, Nishizawa Y, Ishii-Minami N, Akimoto-Tomiyama C, Dohmae N, Takio K, Minami E, Shibuya N (2006) Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. *Proc Natl Acad Sci USA* **103**: 11086–11091
- Lange A, Mills RE, Lange CJ, Stewart M, Devine SE, Corbett AH (2007) Classical nuclear localization signals: definition, function, and interaction with importin alpha. *J Biol Chem* **282**: 5101–5105
- Leipe DD, Koonin EV, Aravind L (2004) STAND, a class of P-loop NTPases including animal and plant regulators of programmed cell death: multiple, complex domain architectures, unusual phyletic patterns, and evolution by horizontal gene transfer. *J Mol Biol* **343**: 1–28
- Ligterink W, Kroj T, zurNieden U, Hirt H, Scheel D (1997) Receptor-mediated activation of a MAP kinase in pathogen defense of plants. *Science* **276**: 2054–2057
- Liu Y, Schiff M, Serino G, Deng XW, Dinesh-Kumar SP (2002) Role of SCF Ubiquitin-Ligase and the COP9 signalosome in the N gene-mediated resistance response to *Tobacco mosaic virus*. *Plant Cell* **14**: 1483–1496
- Liu YL, Burch-Smith T, Schiff M, Feng SH, Dinesh-Kumar SP (2004) Molecular chaperone Hsp90 associates with resistance protein N and its signaling proteins SGT1 and Rar1 to modulate an innate immune response in Plants. *J Biol Chem* **279**: 2101–2108
- Lu R, Malcuit I, Moffett P, Ruiz MT, Peart J, Wu AJ, Rathjen JP, Bendahmane A, Day L, Baulcombe DC (2003) High throughput virus-induced gene silencing implicates heat shock protein 90 in plant disease resistance. *EMBO J* **22**: 5690–5699
- Mayor A, Martinon F, De Smedt T, Petrilli V, Tschoep J (2007) A crucial function of SGT1 and HSP90 in inflammasome activity links mammalian and plant innate immune responses. *Nat Immunol* **8**: 497–503
- McKenna NJ, O'Malley BW (2002) Combinatorial control of gene expression by nuclear receptors and coregulators. *Cell* **108**: 465–474
- Meier I (2007) Composition of the plant nuclear envelope: theme and variations. *J Exp Bot* **58**: 27–34
- Melotto M, Underwood W, Koczan J, Nomura K, He SY (2006) Plant stomata function in innate immunity against bacterial invasion. *Cell* **126**: 969–980
- Merkle T (2001) Nuclear import and export of proteins in plants: a tool for the regulation of signalling. *Planta* **213**: 499–517
- Meyers BC, Kozik A, Griego A, Kuang HH, Michelmore RW (2003) Genome-wide analysis of NBS-LRR-encoding genes in *Arabidopsis*. *Plant Cell* **15**: 809–834
- Moffett P, Farnham G, Peart J, Baulcombe D (2002) Interaction between domains of an plant NBS-LRR protein in disease resistance-related cell death. *EMBO J* **21**: 4511–4519
- Nishimura MT, Stein M, Hou BH, Vogel JP, Edwards H, Somerville SC (2003) Loss of a callose synthase results in salicylic acid-dependent disease resistance. *Science* **301**: 969–972
- Nomura K, DebRoy S, Lee YH, Pumphlin N, Jones J, He SY (2006) A bacterial virulence protein suppresses host innate immunity to cause plant disease. *Science* **313**: 220–223
- Noutoshi Y, Ito T, Seki M, Nakashita H, Yoshida S, Marco Y, Shirasu K, Shinozaki K (2005) A single amino acid insertion in the WRKY domain of the *Arabidopsis* TIR-NBS-LRR-WRKY-type disease resistance protein SLH1 (sensitive to low humidity 1) causes activation of defense responses and hypersensitive cell death. *Plant J* **43**: 873–888
- Palma K, Zhang YL, Li X (2005) An importin alpha homolog, MOS6, plays an important role in plant innate immunity. *Curr Biol* **15**: 1129–1135
- Pemberton LF, Paschal BM (2005) Mechanisms of receptor-mediated nuclear import and nuclear export. *Traffic* **6**: 187–198
- Pratt WB, Toft DO (2003) Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. *Exp Biol Med* **228**: 111–133
- Qiu M, Olsen A, Faivre E, Horwitz KB, Lange CA (2003) Mitogen-activated protein kinase regulates nuclear association of human progesterone receptors. *Mol Endocrinol* **17**: 628–642
- Rairdan GJ, Moffett P (2006) Distinct domains in the ARC region of the potato resistance protein Rx mediate LRR binding and inhibition of activation. *Plant Cell* **18**: 2082–2093
- Ridout CJ, Skamnioti P, Porritt O, Sacristan S, Jones JDG, Brown JKM (2006) Multiple avirulence paralogues in cereal powdery

- mildew fungi may contribute to parasite fitness and defeat of plant resistance. *Plant Cell* **18**: 2402–2414
- Riedl SJ, Li WY, Chao Y, Schwarzenbacher R, Shi YG (2005) Structure of the apoptotic protease-activating factor 1 bound to ADP. *Nature* **434**: 926–933
- Rose A, Meier I (2001) A domain unique to plant RanGAP is responsible for its targeting to the plant nuclear rim. *Proc Natl Acad Sci USA* **98**: 15377–15382
- Serino G, Deng XW (2003) THE COP9 signalosome: regulating plant development through the control of proteolysis. *Annu Rev Plant Biol* **54**: 165–182
- Shank LC, Paschal BM (2005) Nuclear transport of steroid hormone receptors. *Crit Rev Eukaryot Gene Expr* **15**: 49–73
- Shen Q-H, Saijo Y, Mauch S, Biskup C, Bieri S, Keller B, Seki H, Ulker B, Somssich IE, Schulze-Lefert P (2007) Nuclear activity of MLA immune receptors links isolate-specific and basal disease-resistance responses. *Science* **315**: 1098–1103
- Shen QH, Zhou FS, Bieri S, Haizel T, Shirasu K, Schulze-Lefert P (2003) Recognition specificity and RAR1/SGT1 dependence in barley Mla disease resistance genes to the powdery mildew fungus. *Plant Cell* **15**: 732–744
- Shirano Y, Kachroo P, Shah J, Klessig DF (2002) A gain-of-function mutation in an *Arabidopsis* Toll Interleukin-1 receptor-nucleotide binding site-leucine-rich repeat type R gene triggers defense responses and results in enhanced disease resistance. *Plant Cell* **14**: 3149–3162
- Shirasu K, Schulze-Lefert P (2000) Regulators of cell death in disease resistance. *Plant Mol Biol* **44**: 371–385
- Shirasu K, Schulze-Lefert P (2003) Complex formation, promiscuity and multi-functionality: protein interactions in disease-resistance pathways. *Trends Plant Sci* **8**: 252–258
- Stone JM, Liang X, Neel ER, Stiers JJ (2005) *Arabidopsis* AtSPL14, a plant-specific SBP-domain transcription factor, participates in plant development and sensitivity to fumonisin B1. *Plant J* **41**: 744–754
- Takahashi A, Casais C, Ichimura K, Shirasu K (2003) HSP90 interacts with RAR1 and SGT1 and is essential for RPS2-mediated disease resistance in *Arabidopsis*. *Proc Natl Acad Sci USA* **100**: 11777–11782
- Takken FLW, Albrecht M, Tameling WIL (2006) Resistance proteins: molecular switches of plant defence. *Curr Opin Plant Biol* **9**: 383–390
- Tameling WIL, Baulcombe DC (2007) Physical association of the NB-LRR resistance protein Rx with a Ran GTPase activating protein is required for extreme resistance to potato virus X. *Plant Cell* **19**: 1682–1694
- Tameling WIL, Vossen JH, Albrecht M, Lengauer T, Berden JA, Haring MA, Cornelissen BJC, Takken FLW (2006) Mutations in the NB-ARC domain of I-2 that impair ATP hydrolysis cause autoactivation. *Plant Physiol* **140**: 1233–1245
- Tanabe T, Chamaillard M, Ogura Y, Zhu L, Qiu S, Masumoto J, Ghosh P, Moran A, Predergast MM, Tromp G, Williams CJ, Inohara N, Nunez G (2004) Regulatory regions and critical residues of NOD2 involved in muramyl dipeptide recognition. *EMBO J* **23**: 1587–1597
- Tao Y, Xie ZY, Chen WQ, Glazebrook J, Chang HS, Han B, Zhu T, Zou GZ, Katagiri F (2003) Quantitative nature of *Arabidopsis* responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. *Plant Cell* **15**: 317–330
- Thilmony R, Underwood W, He SY (2006) Genome-wide transcriptional analysis of the *Arabidopsis thaliana* interaction with the plant pathogen *Pseudomonas syringae* pv. tomato DC3000 and the human pathogen *Escherichia coli* O157: H7. *Plant J* **46**: 34–53
- Ting JPY, Kastner DL, Hoffman HM (2006) CATERPILLERS, pyrin and hereditary immunological disorders. *Nat Rev Immunol* **6**: 183–195
- Tornero P, Merritt P, Sadanandom A, Shirasu K, Innes RW, Dangl JL (2002) *RAR1* and *NDR1* contribute quantitatively to disease resistance in *Arabidopsis*, and their relative contributions are dependent on the *R* gene assayed. *Plant Cell* **14**: 1005–1015
- Tuskan GA, DiFazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Putnam N, Ralph S, Rombauts S, Salamov A, Schein J, Sterck L, Aerts A, Bhaleerao RR, Bhaleerao RP, Blaudez D, Boerjan W, Brun A, Brunner A, Busov V *et al* (2006) The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* **313**: 1596–1604
- Ulker B, Somssich IE (2004) WRKY transcription factors: from DNA binding towards biological function. *Curr Opin Plant Biol* **7**: 491–498
- van der Biezen EA, Jones JDG (1998) The NB-ARC domain: a novel signalling motif shared by plant resistance gene products and regulators of cell death in animals. *Curr Biol* **8**: R226–R228
- Vasu SK, Forbes DJ (2001) Nuclear pores and nuclear assembly. *Curr Opin Cell Biol* **13**: 363–375
- Walther TC, Askjaer P, Gentzel M, Habermann A, Griffiths G, Wilm M, Mattaj IW, Hetzer M (2003) RanGTP mediates nuclear pore complex assembly. *Nature* **424**: 689–694
- Whitham S, Dinesh-Kumar SP, Choi D, Hehl R, Corr C, Baker B (1994) The product of the tobacco mosaic virus resistance gene *N*: similarity to Toll and the interleukin-1 receptor. *Cell* **78**: 1011–1115
- Wirthmueller L, Zhang Y, Jones JDG, Parker J (2007) Nuclear signaling by the *Arabidopsis* immune receptor RPS4 requires EDS1. submitted
- Xu L, Massague A (2004) Nucleocytoplasmic shuttling of signal transducers. *Nat Rev Mol Cell Biol* **5**: 209–219
- Xu XP, Chen CH, Fan BF, Chen ZX (2006) Physical and functional interactions between pathogen-induced *Arabidopsis* WRKY18, WRKY40, and WRKY60 transcription factors. *Plant Cell* **18**: 1310–1326
- Yamasaki K, Kigawa T, Inoue M, Tateno M, Yamasaki T, Yabuki T, Aoki M, Seki I, Matsuda T, Tomo Y, Hayami N, Terada T, Shirouzu M, Tanaka A, Seki M, Shinozaki K, Yokoyama S (2005) Solution structure of an *Arabidopsis* WRKY DNA binding domain. *Plant Cell* **17**: 944–956
- Yan N, Chai JJ, Lee ES, Gu LC, Liu Q, He JQ, Wu JW, Kokel D, Li HL, Hao Q, Xue D, Shi YG (2005) Structure of the CED-4-CED-9 complex provides insights into programmed cell death in *Caenorhabditis elegans*. *Nature* **437**: 831–837
- Yoshimura S, Yamanouchi U, Katayose Y, Toki S, Wang Z-W, Kono I, Kurata N, Yano M, Iwata N, Sasaki T (1998) Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. *Proc Natl Acad Sci USA* **95**: 1663–1668
- Zhang YL, Li X (2005) A putative nucleoporin 96 is required for both basal defense and constitutive resistance responses mediated by suppressor of *npr1-1*, constitutive 1. *Plant Cell* **17**: 1306–1316
- Zhang YL, Goritschnig S, Dong XN, Li X (2003) A gain-of-function mutation in a plant disease resistance gene leads to constitutive activation of downstream signal transduction pathways in suppressor of *npr1-1*, constitutive 1. *Plant Cell* **15**: 2636–2646
- Zipfel C, Felix G (2005) Plants and animals: a different taste for microbes? *Curr Opin Plant Biol* **8**: 353–360
- Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JDG, Boller T, Felix G (2006) Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. *Cell* **125**: 749–760