PAMP recognition and the plant–pathogen arms race

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Summary

Plants have evolved systems analogous to animal innate immunity that recognise pathogen-associated molecular patterns (PAMPs). PAMP detection is an important component of non-host resistance in plants and serves as an early warning system for the presence of potential pathogens. Binding of a PAMP to the appropriate pattern recognition receptor leads to downstream signalling events and, ultimately, to the induction of basal defence systems. To overcome non-host resistance, pathogens have evolved effectors that target specific regulatory components of the basal defence system. In turn, this has led to the evolution in plants of cultivar-specific resistance mediated by R proteins, which guard the targets of effectors against pathogen manipulation; the arms race continues. ***BioEssays*** **28:880–889, 2006.**

Introduction

Disease is actually a relatively rare phenomenon in plants; the majority of plant species are resistant to infection by all isolates of any given microbial species.[1,2] The ability of an entire plant species to resist infection by all isolates of a pathogen species is termed non-host (or species) resistance. This is the commonest form of disease resistance in plants, and the infrequent change in the range of host species colonised by plant pathogens is indicative of its stability.[2,3]

Non-host resistance is thought to rely on both pre-formed barriers, such as the waxy cuticle and cell wall, which physically impede the growth and spread of the potential pathogen, and on the induction of the basal defence system mounted in response to the recognition of non-self by the plant.[1,2,4] An array of microbial-derived molecules termed pathogen-associated molecular patterns (PAMPs) are recognised by pattern recognition receptors (PRRs) in the plant leading to signal transduction and the activation of a range of basal defence mechanisms including ethylene (ET) production, an oxidative burst, callose deposition, induction of defence-related gene expression and, in some cases, hypersensitive response (HR)-like cell death.[5–8] Induction of these defence mechanisms also occurs in host plants in response to virulent pathogens (sometimes termed basal resistance) but is ineffective at controlling the growth of the pathogen. As non-host and basal resistance utilise the same defence mechanisms, non-host resistance may represent successful control of pathogen growth by the basal defence systems, while host susceptibility results from ineffective induction or pathogen suppression of these systems (Fig. 1A–C).

The generation of pathogen strain-specific antibodies via somatic recombination (adaptive immunity) in animals, vertebrates specifically, is well known. However, animals have a second surveillance system for detecting non-self—PAMP detection by PRRs termed innate immunity. The PAMP detection system present in plants corresponds conceptually to that of the innate immune system in animals; both recognise highly conserved microbial molecules and act as an early warning system for the presence of a potential pathogen.[9] Plants also have a second system, cultivar-specific resistance, involving pairs of gene products—effector molecules from the pathogen and corresponding resistance (R) proteins in the plant. Recognition of an effector, or of its activity, by the appropriate R protein in the host leads to the HR and curtailment of pathogen growth, while loss of either of these proteins results in disease.[1] Since effectors are specific to particular pathogen strains, it has been proposed that cultivar-specific resistance fulfils an analogous role in plants to that of the adaptive immune system in vertebrates.[9,10] However,

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Abbreviations: EF-Tu, elongation factor Tu; ET, ethylene; flg22, flagellin epitope; FLS2, flagellin receptor; GSNO, glutathione *S*-nitrosothiol; GSNOR, glutathione *S*-nitrosothiol reductase; HR, hypersensitive response; JA, jasmonic acid; LPS, lipopolysaccharide; LRR, leucine-rich repeat; MAPK, Mitogen-activated protein kinase; NO, nitric oxide; PAMP, pattern-associated molecular pattern; PRR, pattern recognition receptor; Pst, *Pseudomonas syringae* pv. *tomato* DC3000; R protein, resistance protein; RLK, receptor-like kinase; ROS, reactive oxygen species; SA, salicylic acid; SNO, *S*-nitrosothiol; TLR, Toll-like receptor; TTSS, type III secretion system.

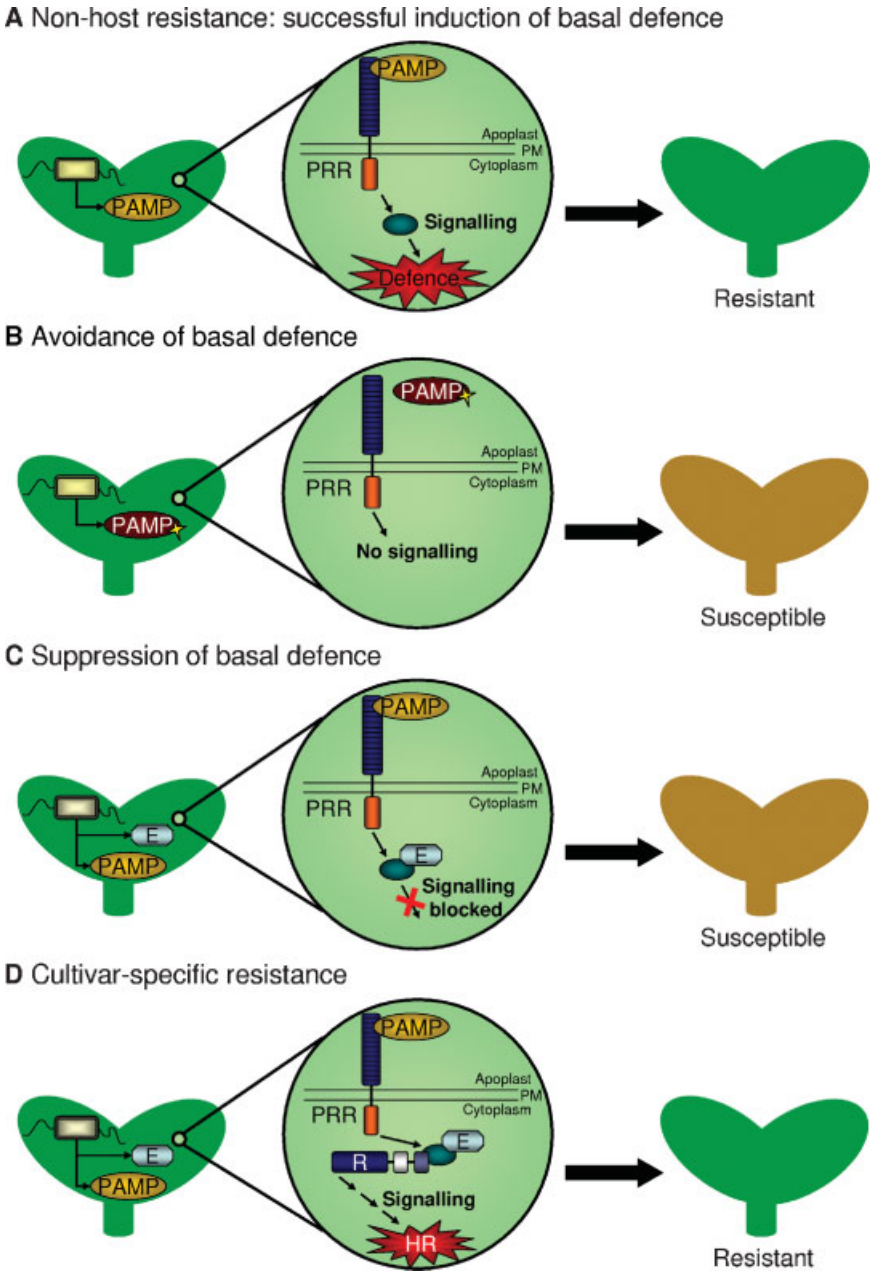

Figure 1. The evolution of plant disease resistance: the proposed relationship between non-host and cultivar-specific resistance. **A:** Non-host resistance: bacteria enter the plant and recognition of a PAMP by the corresponding PRR results in downstream signal transduction and the activation of basal defence mechanisms. Successful induction of the basal defence systems renders the plant resistant to pathogen colonisation. **B:** A possible mechanism for overcoming non-host resistance in plants is the evolution of non-eliciting PAMPs, which are not recognised by the specific plant PRR. Basal defence systems are not activated and the plant is susceptible to infection. **C:** The evolution of effector proteins (E) in pathogens is an alternative strategy for overcoming non-host resistance. Effectors target signalling components regulating the basal defence system to suppress the defence response downstream of PAMP recognition. The plant is then susceptible to disease. **D:** The evolution of effectors in pathogens consequently led to the evolution of cultivar-specific resistance in plants. To overcome the suppression by effectors, certain cultivars of a susceptible plant species evolved R proteins that recognise the activity of the corresponding effector molecule. Recognition leads to the HR preventing further pathogen colonisation.

importantly the plant resistance is identical within all individuals of a cultivar whereas adaptive immunity differs between individual animals. It is therefore generally thought that cultivar-specific resistance is also a form of innate immunity and that plants lack adaptive immune systems.[2–5,9] The evolution of effectors in pathogens is believed to have allowed individual strains to overcome non-host resistance in plants through avoidance or suppression of the basal defence systems.[11,12] This in turn led to the evolution of cultivar-specific resistance where only certain cultivars (those possessing the appropriate *R* gene) of an otherwise susceptible plant species display resistance to a given pathogen (Fig. 1C,D).[3]

In this review, we will focus on recent studies of PRR-mediated recognition of PAMPs and subsequent signalling events that lead to the activation of basal defence systems in plants, using the flagellin perception system as a model. We will also consider new evidence indicating that effectors from pathogens specifically target key components of the basal defence system to overcome non-host resistance, and that these targets are in turn guarded by R proteins in the continuing arms race between plants and pathogens.

Recognising non-self

The ability to determine self from non-self is critical for plants to mount an effective immune response against potential pathogens. PAMPs, also known as general elicitors, offer one such opportunity. PAMPs are highly conserved and ubiquitous molecules widely distributed amongst microbial species (pathogenic or not) where they carry out an essential function, but absent in the potential host species.[2,13] A number of PAMPs that fulfil these criteria and elicit a defence response in plants have been identified from plant pathogens (comprehensively reviewed in Nürnberger et al.[14] and Nürnberger and Lipka[2]) including flagellin, cold-shock protein, lipopolysaccharide (LPS) and elongation factor Tu (EF-Tu) from Gram-negative bacteria, and chitin, β-glucans and ergosterol from fungi. Different plant species respond to different PAMPs e.g. tobacco responds to cold-shock protein while Arabidopsis does not, and only members of the Brassicaceae have so far been shown to respond to EF-Tu.[5,7]

While this represents a diverse set of molecules, within the proteinaceous PAMPs two themes have emerged. These molecules typically contain a short (10–25) amino acid epitope that elicits a stronger defence response than the complete protein. For example, from Gram-negative bacteria flg22, a highly conserved stretch of 22 amino acids from the N terminus of flagellin, is a more potent elicitor than flagellin,[6] and the same is true of a highly conserved 15 amino acid stretch including the RNA-binding motif RNP-1 from the cold-shock protein[5] and an 18 amino acid stretch from the N terminus of the elongation factor EF-Tu.[7] Similarly, the fungal elicitor Pep-13 is a 13 amino acid internal peptide of a 42 kDa transglutaminase enzyme from the cell wall of *Phytophthora sojae*.[8,15] However, there are exceptions; the elicitor effect of NPP1 (necrosis-inducing Phytophthora protein 1) requires an intact protein and overlapping peptide fragments were inactive,[16] perhaps indicating that it is the activity of this protein that is detected by the plant rather than a specific amino acid sequence.

Presumably, there would be a huge selective advantage for mutations within these epitopes that rendered them inactive as elicitors of plant defence systems. However, it would seem that, in many cases, such mutations also have a deleterious effect on the function of these proteins in the pathogen. For example, in Pep-13, substitution of Trp^{231} to Ala abolished elicitor activity in parsley but with a concurrent 98% reduction in transglutaminase activity.[15] Similarly, substitutions within the RNP-1 motif of the cold-shock protein that led to a reduction in elicitor activity also had a negative effect on the RNA-binding affinity of this protein.[5] Thus, it appears that plants have evolved receptors that recognise short highly conserved amino acid stretches of certain microbial proteins that cannot easily be altered without loss of the protein function. That said, certain microbes may have evolved the capacity to avoid detection by specific PRRs. For example, *Agrobacterium tumefaciens* and *Ralstonia solanacearum* (pathogens) as well as *Rhizobium meliloti* (symbiont) possess functional flagellins that do not elicit a defence response in Arabidopsis and the N-terminal peptide from *Pseudomonas syringae* pv. *tomato* DC3000 (Pst) EF-Tu is not as potent an elicitor in Arabidopsis as those from other bacteria.[6,7,17] In *Xanthomonas campestris* pv. *campestris* a single valine/aspartate polymorphism within the flg22 peptide determines eliciting ability of this molecule.[17] The evolution of non-eliciting PAMPs is one way in which pathogens can overcome non-host resistance in plants; however, the lack of a single eliciting PAMP has not yet been directly shown to affect the virulence of the pathogen. Some experiments have shown that deletion of a specific PRR in the host affects susceptibility; however, in other studies wild-type plants and plants lacking a PRR were equally susceptible.[17–19] This could be explained by the evolution in plants of recognition systems for multiple PAMPs from the same micro-organism. For example, Arabidopsis recognises both flagellin and EF-Tu and these PAMPs activate the same signalling and defence responses in a non-synergistic manner.[18] A recent gene expression profiling study has also demonstrated that the lack of flagellin perception does not dramatically alter PAMP-induced gene expression during infection of Arabidopsis by Pst.[20]

PAMP detection

Although numerous PAMPs have been characterised from micro-organisms, identification of the corresponding PRRs from plants has lagged behind, and evidence for these

receptors comes primarily from biochemical data. Binding proteins for several PAMPs have been characterised in chemical cross-linking studies, e.g. 100 kDa and 145 kDa proteins from parsley membranes bind Pep-13[21] and 162 kDa and 50 kDa proteins from tobacco bind cryptogein,[22] but isolation of genes encoding such putative receptors has proved problematic. There are several notable exceptions. Glucan-binding protein from soybean consists of a glucan hydrolase domain and a β-glucan-binding domain. This soluble protein lacks any known signalling motifs and has been postulated to function as part of a receptor complex.[23] The tomato receptor for the fungal elicitor ethylene-induced xylanase is a membrane-spanning protein with leucine zipper and leucine-rich repeat sequence (LRR) extracellular domains. The intracellular domain contains a mammalian endocytosis signal which if mutated abolishes the elicitor-induced HR.[24] The best-studied PRR is the flagellin receptor gene *FLS2* (flagellin sensitive 2) identified in a forward-genetics screen for mutants unable to respond to the flg22 epitope.[25] *FLS2* encodes a 120 kDa receptor-like kinase (RLK), and was recently shown to bind the flg22 epitope.[26] RLKs consist of a signal peptide, an extracellular ligand-binding domain, a single membrane-spanning region and an intracellular serine/threonine kinase domain.[27] Some 620 RLK-like sequences are present in the Arabidopsis genome, of which those containing a LRR in the extracellular domain constitute the largest group with 216 members including FLS2.[27] Recently, another member of this group was identified as the receptor for bacterial EF-Tu.[18] Arabidopsis *efr* (EF-Tu receptor) mutants display increased transformation efficiency by *A. tumefaciens* demonstrating the importance of this PAMP recognition in defence.[18]

In mammals a family of conserved transmembrane Toll-like receptors (TLRs) function directly or indirectly as PRRs for PAMPs.[28] These receptors contain an extracellular LRR and an intracellular TIR domain (named after Drosophila *T*oll and human *i*nterleukin-1 *r*eceptors). Different TLRs recognise different PAMPs; TLR5 recognises flagellin, TLR4 LPS and TLR9 bacterial unmethylated CpG DNA.[29–31] It is likely that an analogous situation exists in plants; Arabidopsis *fls2* mutants are still able to respond to EF-Tu and *efr* mutants respond to flg22.[7,18] Animals possess a limited set of TLRs and so it has been suggested that heterodimerisation or the recruitment of extracellular adaptor proteins increases the number of PAMPs that can be detected. For example, TLR2 forms a heterodimer with TLR6 to detect zymosan and peptidoglycan[32] while TLR4 requires the extracellular protein MD2 for both LPS and taxol recognition.[33] It is possible that similar mechanisms occur in plants to increase the range of PAMPs that can be detected but there is no strong evidence for this to date. Furthermore, as plants possess a much larger number of potential receptors in their genomes than animals, with 216 LRR RLKs in Arabidopsis compared to 10 TLRs in humans,[27,28] it is possible that such mechanisms may not be required.

It has been proposed that the innate immune systems present in plants and animals diverged from an ancient system present in their last common ancestor. This view is based mainly on the fact that both FLS2 and TLR5 possess an extracellular LRR involved in ligand binding, which leads to activation of a serine/threonine protein kinase. However, there is little sequence similarity between the LRRs of FLS2 and TLR5 and they recognise different epitopes of flagellin.[34,35] In addition, the intracellular domains show no homology; FLS2 has an intracellular serine/threonine kinase domain,[25] while the TIR domain of TLR5 recruits serine/threonine kinases of the IRAK family through the adaptor protein MyD88.[29] Thus there is no conclusive evidence for evolutionary conservation of an ancient PAMP detection system, and it is equally plausible that these components were recruited independently during convergent evolution.[9,36]

PAMP signal transduction

Downstream of PRRs several components of the signalling network mediating PAMP-induced responses have been identified. Here we focus on recent work that has contributed to the understanding of signal cascades triggered by PAMPs. The most-important finding has been the discovery of significant overlap between components involved in transducing signals in response to PAMP detection and those involved in cultivar-specific resistance mediated by R proteins.

One such component is nitric oxide (NO). AtNOS1, a plant-specific NO synthase, mediates LPS-induced NO production and pathogenesis released *(PR)* gene expression in Arabidopsis.[37] In addition, NO production increased in tobacco plants challenged with a non-host *P. syringae* pathogen.[38] In cultivar-specific resistance, infection with avirulent *P. syringae* causes rapid accumulation of NO in Arabidopsis leaves and inhibition of NO accumulation during pathogen infection led to a reduction in the HR and defence gene expression.[39]

Biological activity of NO can also be mediated by *S*-nitrosylation of cysteine residues. The production of protein nitrosothiols (protein SNOs) can alter the protein activity, whereas the formation of glutathione nitrosothiols (GSNOs) is thought to provide a reservoir of biologically active NO metabolites. *S*-nitrosoglutathione reductase (GSNOR) metabolises GSNOs and appears to regulate levels of protein *S*-nitrosylation. In Arabidopsis, protein SNO levels were decreased in plants with enhanced GSNOR activity, and increased in plants with a loss-of-function mutation in *AtGSNOR1*. Protein SNO levels may play a role in the regulation of defence responses as disease resistance was increased in the plants with enhanced GSNOR activity and compromised in plants with reduced GSNOR function.[40] Importantly, the change in defence phenotype affected both non-host and R protein-mediated resistance.

Mitogen-activated protein kinase (MAPK) cascades are another component used in transducing both non-host and R protein-mediated signals. These cascades are signalling modules involving sequential transfer of phosphate groups from a MAPK kinase kinase (MAPKKK), to a MAPK kinase (MAPKK) to a MAPK and onto downstream targets. A common role for MAPKs in PAMP- and R protein-mediated resistance had been suggested by the observation that two tobacco MAPKs (SIPK and WIPK) are activated by tobacco mosaic virus and some R-effector protein interactions as well as by flagellin and fungal cell wall-derived elicitors.[41,42] A more recent study has shown that Arabidopsis MPK6 (a MAPK) is required for the activation of both basal defence systems and cultivar-specific resistance.[43] Silencing of MPK6 resulted in increased growth of virulent and avirulent isolates of *P. syringae* and of an avirulent isolate of *Hyaloperonospora parasitica*.

Salicylic acid (SA), jasmonic acid (JA) and ET are well-documented as playing a role in both basal and cultivar-specific resistance.[44–46] Here we will specifically discuss their involvement in defence against non-host pathogens. Cowpea rust fungus, a non-host pathogen on Arabidopsis, was able to cause disease on the *sid2* (salicylic acid induction-deficient 2) mutant.[47] *Sid2* is unable to synthesize SA suggesting that this compound is important in regulating non-host resistance. Similarly, the barley powdery mildew was able to grow in Arabidopsis *NahG* transgenics which are unable to accumulate SA.[48] However, expression profiling of Arabidopsis after challenge with non-host pathogens has suggested that the JA/ET pathway is playing a more significant role in non-host resistance. Infection with the non-host pathogen *Phytophthora infestans* resulted in a pattern of gene expression that was most similar to that of methyljasmonate treatment.[49] Furthermore, the role of SA in flagellin perception is unclear. Mutant phenotypes suggest that flg22-induced resistance is independent of SA, JA and ET.[19] However, in wild-type plants flg22 induces expression of *PR1* (a SA marker gene) and production of ET.[6,50] One potential explanation is that this PAMP might activate all three signalling pathways in parallel and hence blocking of a single pathway does not dramatically affect resistance.

During the past years, several key players in both non-host and cultivar-specific resistance have been cloned and characterized. Arabidopsis EDS1 (enhanced disease susceptibility 1) and its interacting partner PAD4 (phytoalexin deficient 4) are essential in cultivar-specific resistance mediated by the TIR-NB-LRR class of R proteins.[51,52] EDS1 and PAD4 have additional roles in non-host resistance since Arabidopsis *eds1* and *pad4* mutants showed increased growth of a wheat and a barley powdery mildew.[48,53] The simultaneous loss of EDS1 and actin cytoskeleton function particularly affected non-host resistance as the wheat pathogen could undergo asexual reproduction with formation of conidiophores in addition to proliferation of hyphae.[53] A recently identified EDS1 interactor, SAG101 (senescence-associated gene 101), has an overlapping function to PAD4 in cultivar-specific resistance.[54] These two proteins appear to function in a similar way in non-host resistance. Challenged with non-host powdery mildew isolates, the *pad4 sag101* double mutant displayed a loss of non-host resistance exceeding that of the single mutants *eds1, pad4* and *sag101*.[55] The shared activities of PAD4 and SAG101 thus represent an important obstacle to both host and non-host pathogens.[55,56]

Protein degradation also plays a role in both non-host and cultivar-specific resistance. SGT1 is a highly conserved component of the plant SCF (Skp1-Cullin-F box protein) ubiquitin ligase complex involved in the degradation of negative regulators of R protein-dependent defence pathways.[57–59] The silencing of *SGT1* in *Nicotiana benthamiana* highlighted its additional function in non-host resistance as several non-host pathogens could grow in silenced plants.[60] Recently MOS3 (modifier of *snc1, 3*) has been identified as a nucleoporin-like protein with a role in the signal transduction pathways regulating basal defence systems and cultivar-specific resistance.[61] It will be interesting to specifically test the function of this protein in defence against a non-host pathogen to confirm that cytoplasmic-nuclear trafficking is another shared component between PAMP- and R protein-mediated defence.

An example: flagellin perception in plants

Flagellin is the main component of the flagellar filament of eubacteria and its perception is the best-characterized PAMP detection system to date in plants. Fig. 2 depicts a model of our current understanding of flagellin detection and signalling in plants.[10,62,63] Flg22 interacts with the extracellular LRR domain of the FLS2 receptor.[26,64] In addition to the role of the kinase domain in subsequent signal transduction,[65] autophosphorylation of FLS2 is essential for ligand binding.[66] One possible mechanism for regulating activity of the FLS2 receptor also involves changes in phosphorylation status. The kinase-associated protein phosphatase, KAPP, interacts with the kinase domain of FLS2 and studies with KAPP-over-expressing plants suggest it acts as a negative regulator of the flagellin signal transduction pathway by keeping the FLS2 kinase domain in an inactive dephosphorylated state.[66] Recently, ligand-mediated receptor endocytosis has been identified as an additional FLS2 regulatory mechanism.[65] After binding of flg22, FLS2 accumulates in mobile intracellular vesicles. This ligand-induced FLS2 endocytosis is followed by receptor degradation possibly via lysosomal and/or proteasomal pathways. Endocytosis and downstream signalling are closely linked but it is not yet known if the actual internalisation is required for signal transduction.

Cellular responses to flagellin include cytosolic and nuclear calcium fluxes,[67] medium alkalinization and the production of

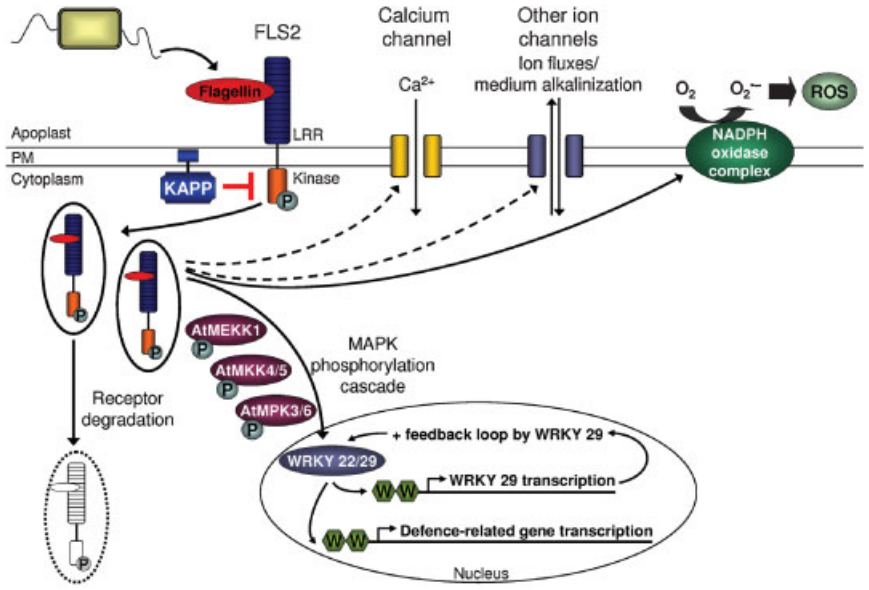

Figure 2. A current model for flagellin perception and signalling in plants. Flagellin interacts with the extracellular LRR domain of the FLS2 receptor. Autophosphorylation of the intracellular FLS2 kinase domain is crucial for ligand binding as well as signalling. KAPP acts as a negative regulator of the flagellin signal transduction pathway. After ligand binding, FLS2 accumulates in mobile intracellular vesicles and is then degraded. Calcium fluxes in response to flagellin include increases in free calcium concentration in the nucleus and in the cytosol. Plasma membrane calcium channels are illustrated but the source of the calcium increase has not yet been determined. Other cellular responses to flagellin include medium alkalinization and the production of ROS through the NADPH oxidase complex. Dashed arrows indicate flagellin-induced responses for which the requirement of flagellin binding has not been directly shown. FLS2 kinase activity is required for the activation of a flg22-responsive MAPK cascade. The phosphorylated AtMEKK1 phosphorylates AtMKK4 and AtMKK5 that phosphorylate and activate AtMPK3 and AtMPK6 leading to expression of the transcription factors WRKY22 and WRKY29. WRKY22 and WRKY29 regulate expression of flagellin-induced defence genes. WRKY29 is also involved in signal amplification through positive feedback on its own expression.

reactive oxygen species (ROS) through the NADPH oxidase complex.[6] However, only the generation of ROS has been shown to require flg22 binding to a functional FLS2 receptor.[65] FLS2 kinase activity is required for the activation of a flg22-responsive MAPK cascade. Evidence from expression of tagged proteins and dominant negative mutants suggests the following sequence of events: phosphorylated and activated AtMEKK1 (a MAPKKK) phosphorylates AtMKK4 and 5 (MAPKKs) that in turn phosphorylate and activate the MAPKs AtMPK3 and AtMPK6.[62] This cascade culminates in the expression of the key defence transcription factor WRKY29 (and probably WRKY22) which is thought to regulate expression of flagellin-induced genes such as *PR1* and *PR5*.[50,62] WRKY29 activates expression from its own promoter leading to signal amplification through a positive feedback loop.[62]

The arms race: targeting of the basal defence system by pathogen effectors

Induction of basal defence mechanisms occurs in response to PAMPs in both host and non-host plant species, so clearly it is not always sufficient to control pathogen growth. For example, Pep-13 from *P. infestans* elicits similar defence responses in parsley and potato, yet parsley is resistant to infection while potato is not.[8] Similarly flagellin from Pst elicits a basal defence response in Arabidopsis that is not sufficient to prevent colonisation by the pathogen.[19] As discussed above, one way for potential pathogens to circumvent non-host resistance is to evolve PAMPs that can no longer be detected by the plant (Fig. 1B). An alternative strategy is to evolve mechanisms for the active suppression of plant basal defences (Fig. 1C), and there is a considerable volume of evidence to suggest that this has occurred in many successful plant pathogens with the evolution of effector molecules.[2,11]

The first study to demonstrate active suppression of basal defence responses in plants by virulent bacteria was carried out by Jakobek et al.[68] who showed that an avirulent strain of *P. syringae* pv. *tabaci* led to an increase in defence-related gene expression and phytoalexin synthesis in beans, whereas infiltration with a virulent strain of *P. syringae* pv. *phaseolicola* did not. Pre-exposure of the plant to the virulent strain for 6 h prior to infiltration with the avirulent strain led to a significant reduction in induction of gene expression, suggesting that the virulent strain was able to suppress activation of basal

defence systems. Numerous studies have since demonstrated that the ability to suppress the basal defence system requires the delivery of effectors into the plant cell by the Type III secretion system (TTSS).[11] For example, virulent *X. campestris* strains were able to suppress the induction of callose deposition in pepper elicited by LPS, while *hrcC*⁻ mutants that lack one of the structural proteins of the TTSS were not.[69] Similarly, expression of the effectors AvrRpt2 or AvrRpm1 in Arabidopsis suppresses the induction of callose deposition and *GST6* expression triggered by a TTSS-deficient *P. syringae* strain or flg22.[70] Microarray analysis of gene expression profiles in Arabidopsis after infiltration with the virulent Pst or a *hrpS*⁻ mutant (another TTSS mutant) identified 117 genes that were differentially expressed. These included a set of genes encoding putative secreted cell wall and defence proteins that were down-regulated in tissue infected with the wild-type strain compared to the TTSS mutant strain. Heterologous expression of the *P. syringae* effector AvrPto led to the repression of a similar set of genes, abolition of the callose deposition normally observed in response to the *hrpS*⁻ mutant and increased colonisation of the plant by this TTSS mutant.[71] Two recent expression profiling studies have identified a large number of PAMP-induced genes in Arabidopsis. Many of these were repressed or not induced after infection with virulent Pst.[20,72] One study provided further strong evidence of suppression of basal defence gene induction by TTSS effectors by comparing expression profiles between COR⁻ and COR⁻*hrpS*⁻ bacterial mutants. This comparison specifically targeted regulation by effector proteins and identified more than 500 genes that were repressed in an effector-dependent manner.[20] In both studies, many of the genes suppressed by virulent bacteria have a role in signal transduction such as RLKs (including FLS2) and WRKY transcription factors.[20,72]

Direct evidence that pathogen effectors are capable of suppressing basal defences comes from a study of Arabidopsis NONHOST1 (NHO1), a glycerol kinase required for non-host resistance against avirulent *Pseudomonas* bacteria.[73] NHO1 is upregulated after exposure to avirulent *Pseudomonas* strains but only transiently induced after infection with the virulent Pst. Once again, a functional TTSS system was required for suppression of this defence response. Recently Li et al.[74] demonstrated that upregulation of *NHO1* could be attributed to the perception of flagellin. Non-host *Pseudomonas* strains lacking flagellin did not induce *NHO1* expression and were able to colonise Arabidopsis and cause disease symptoms. Most importantly, expression of nine different effectors in the host, including AvrPto, was able to suppress flagellin-induced *NHO1* expression.[74]

Thus there is strong evidence that successful colonisation of several plant species by virulent bacterial strains is in part mediated by targeting of components of the basal defence system by effectors delivered via the TTSS. In turn, evolution of R proteins has provided a mechanism for plants to monitor pathogen effector activity (Fig. 1D). Van der Biezen and Jones[75] formulated the 'guard hypothesis' that proposed that R proteins guard the targets of Type III effectors, which include components of the basal defence system. They suggested that rather than directly interacting with effector molecules (little evidence for this had been forthcoming), R proteins formed complexes with the targets of effectors, and were activated upon modification or degradation of these targets leading to the HR and cultivar-specific resistance. Recent studies of RIN4, a membrane-associated protein in Arabidopsis, have provided substantial evidence to support this hypothesis. RIN4 acts as a negative regulator of PAMP-mediated basal defence responses (Fig. 3A,B) as its overexpression in Arabidopsis led to a reduction in callose deposition and *GST6* induction after exposure to flagellin or a normally non-pathogenic *P. syringae hrcC*⁻ mutant, and allowed the *hrcC*⁻ mutant to multiply.[70] Conversely *rin4* plants show reduced growth of the *hrcC*⁻ mutant with increased activation of callose deposition and *GST6* expression.[70] This component of the basal defence system is targeted by at least three effectors from *P. syringae*: AvrB, AvrRpm1 and AvrRpt2.[70,76] Heterologous expression of AvrRpm1 and AvrRpt2 gives the same phenotype as RIN4 overexpression. AvrRpm1 (and AvrB) induce phosphorylation of RIN4, which may enhance its inhibitory effect on basal defence activation (Fig. 3C).[76] AvrRpt2 is a cysteine protease that cleaves RIN4 leading to its degradation by the proteasome.[77] As predicted by the guard hypothesis, RIN4 is guarded by two R proteins (RPM1 and RPS2), which detect effector-mediated perturbations to RIN4 function. Phosphorylation of RIN4 is thought to lead to activation of RPM1 and induction of the HR, rendering AvrRpm1-carrying strains avirulent (Fig. 3D).[76] Reduction of RIN4 levels also leads to a decrease in RPM1 accumulation and it has been suggested that the AvrRpt2-mediated degradation of RIN4 may have evolved as a way for pathogen strains carrying the AvrRpm1 effector to avoid detection (Fig. 3E).[77] Indeed, degradation of RIN4 by AvrRpt2 blocks plant recognition of AvrRpm1 but if the host expresses a non-cleavable RIN4 protein, AvrRpt2 cannot prevent AvrRpm1 recognition.[77] A similar scenario may explain the ability of various *P. syringae* effectors to suppress the HR induced by the presence of another *P. syringae* effector, HopPsyA.[78] In the case of RIN4, the arms race may have continued with the subsequent evolution of the RPS2 R protein in plants, enabling the detection of AvrRpt2-mediated RIN4 degradation and induction of HR once again (Fig. 3F). RIN4 is thus a mechanistic and physical link between PAMP-mediated and R protein-mediated plant defence responses. The formation of complexes between R proteins and components of the basal defence system may be a common strategy to detect effector activity in plants, and represents an alternative strategy to the adaptive immune

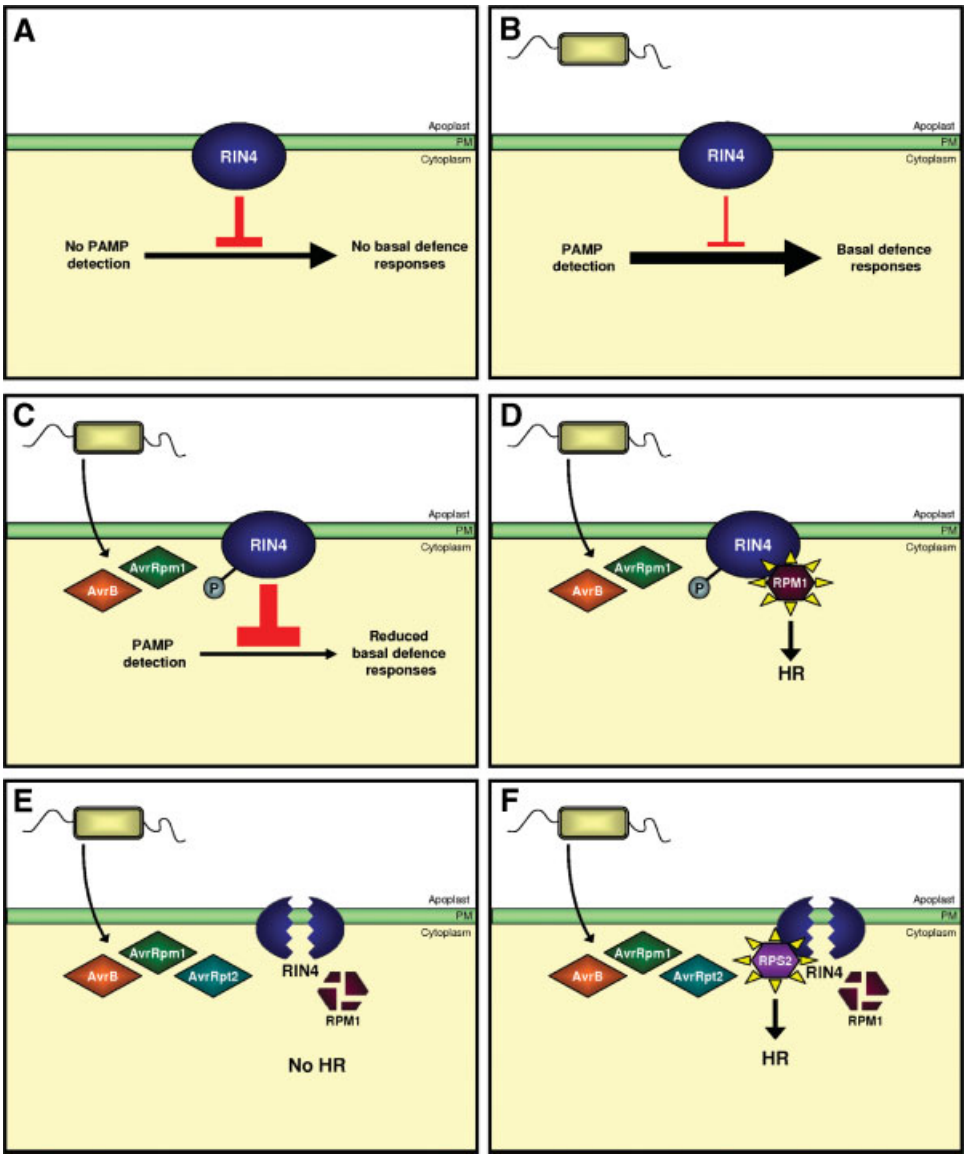

Figure 3. Proposed action of RIN4 during basal and R protein-mediated defence. **A:** RIN4 is a negative regulator of basal defence. **B:** After PAMP detection activation of basal defence must overcome or suppress RIN4 inhibition to allow induction of defence responses. **C:** Phosphorylation of RIN4 induced by the effectors AvrB and AvrRpm1 is thought to enhance RIN4's inhibitory effect resulting in reduced activation of basal defence responses and enabling pathogen growth. **D:** When present, the R protein RPM1 is thought to detect phosphorylation of RIN4 and activates the HR preventing pathogen spread. **E**. The evolution of the AvrRpm2 effector may have enabled pathogens carrying AvrB/AvrRpm1 to avoid RPM1-mediated detection as degradation of RIN4 by AvrRpt2 also leads to decreased accumulation of RPM1. **F:** Plants have evolved a second R protein (RPS2) to guard RIN4. RPS2 is activated when RIN4 is degraded by AvrRpt2 again leading to the HR and cultivar-specific resistance.

system of animals for the detection of specific pathogen strains.[9]

Conclusion

PAMP detection is now known to play an important role in the activation of basal defence systems in plants and as a component of non-host resistance. The importance of basal defence in plants is underlined by the evolution of effectors in pathogens which suppress induction of these systems allowing successful pathogen colonisation and growth. A significant number of PAMPs have been identified but the identification of the PRRs that detect them lags behind and remains one of the major challenges in the study of

plant–pathogen interactions. Downstream of PAMP detection, it has emerged that many signalling components are required for the activation of both basal defence systems and R protein-mediated resistance. However, it is possible that some of these signalling components may be the targets of pathogen effectors and hence guarded by R proteins and only acting as true signalling components in non-host resistance. Further investigation of the targets of effectors should help to resolve this question, and also aid in the identification of additional components of the basal defence system in plants.

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