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News from the frontline: recent insights into PAMP-triggered immunity in plants

Benjamin Schwessinger and Cyril Zipfel

Plants have developed a complex defence network to fight off invading pathogens. In recent years, the full importance of pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) within this network became apparent. Several new PAMPs have been isolated and new pattern-recognition receptors (PRRs) identified. The discovery of the PRR-interacting protein BAK1 sheds light on the immediate downstream signalling events. Further, transcriptomic analyses identified a core set of ~100 PAMP-responsive genes. These studies also revealed a significant overlap with genes regulated during effector-triggered immunity (ETI). Strikingly, ETI seems to operate by alleviating the negative feedback regulation of PTI, leading to stronger defences. This review discusses recent findings in PTI recognition and signalling, and illustrates the need to discover new regulators of PTI responses for a full understanding of plant innate immunity.

Addresses
The Sainsbury Laboratory, Colney Lane, Norwich NR4 7UH, UK

Corresponding author: Zipfel, Cyril (cyril.zipfel@tsl.ac.uk)

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Plants are constantly surrounded and attacked by a plethora of microorganisms including plant pathogens. The first line of active defence relies on the recognition of pathogen-associated (or microbe-associated) molecular patterns (PAMPs/MAMPs) by PRRs leading to PTI [1••,2••]. The importance of PTI becomes apparent by enhanced susceptibility of mutants in PTI recognition or signalling components to adapted or non-adapted bacteria. Furthermore, successful pathogens need to suppress PTI using effectors or toxins to achieve their full virulent potential [3•,4••] (reviews by Alfano, Hogenhout, Birch and Hussey, this issue). PTI suppression together with other effector functions leads to effector-triggered susceptibility (ETS) [1••]. Some plants have evolved to sense specific pathogen effectors by R-proteins leading to ETI. Often ETI is quantitatively stronger than PTI and is accompanied by a hypersensitive response form of programmed cell death [1••].

In this review we will focus on recent progress in the identification of new molecules activating PTI, new insights into PAMP recognition and downstream signalling. We will also discuss the role of negative feedback regulation and the interconnection of PTI and ETI signalling.

PAMPs/MAMPs/DAMPs: the thing that gives you away

PAMPs/MAMPs are defined as invariant epitopes within molecules that are fundamental to the pathogens' fitness, widely distributed among different microbes, absent in the host and recognised by a wide array of potential hosts.

Filamentous oomycetes and fungi

Pep13 was the first molecule clearly defined as a PAMP. This is a short 13-amino acid peptide of a conserved surface-exposed fragment within a calcium-dependent cell-wall transglutaminase from the oomycete *Phytophthora sojae* that elicits defence responses in *Solanaceae* [5]. Recently, the cellulose-binding elicitor lectin from *Phytophthora parasitica* var. *nicotianae* was shown to trigger defence responses in *Arabidopsis* and tobacco [6]. The two cellulose-binding domains, which are probably important for cell adhesion during infection, are sufficient for defence induction. Additionally, many plant-species-specific elicitors from oomycetes are known such as elicitins and heptaglucosides [5].

Other more broadly recognised fungal PAMPs are derived from cell-wall components, such as β-glucan, ergosterol or chitin [5].

Bacteria

Plants recognise many different bacterial PAMPs. Lipopolysaccharides (LPSs) are perceived by a range of plant species [7]. The main PAMP of LPS is the widely conserved lipid A. However, the core oligosaccharide and O-antigen structure are also recognised, potentially by a different perception system than lipid A in *Arabidopsis* [7]. Peptidoglycans (PGNs) are a major cell-wall component of Gram-positive bacteria. PGN sugar chains longer than the disaccharide, but not the protein moieties, are recognised as a PAMP in *Arabidopsis* [8]. The highly conserved N-terminal domain of flagellin (flg22) is another extracellular PAMP which is recognised by most plant species [9]. Tomato is able to recognise a shorter version of the same epitope (flg15) and rice

seems insensitive to flg22 but can recognise full-length flagellin [10,11•].

In addition, several bacterial pathogens can evade flagellin recognition. For example, *Xanthomonas campestris* pv. *campestris* (*Xcc*) has within-species polymorphism in flg22, which leads to the inability of *Arabidopsis* to recognise the 'disguised' epitope [12•], though the biological relevance of this relative recognition on *Xcc*-adapted hosts needs further investigation. Therefore, PAMPs are more variable than previously anticipated and the recognised sub-molecular epitopes can vary between plant species.

Notably, not all PAMPs are unambiguously part of extracellular entities. The bacterial cold shock protein (CSP) and the translation elongation factor Tu (EF-Tu) are best known as intracellular proteins. Nevertheless, the core 22-amino acid (csp22) of the RNA-binding domain-1 and the *N*-acetylated N-terminal 18 amino acids of EF-Tu (elf18) are recognised by *Solanaceae* and *Brassicaceae*, respectively [9].

Overall the variability of PAMP recognition between different plant species demonstrates a diversity of PRR specificities. This diversity can be potentially exploited by pyramiding PRRs or chimeric PRRs of orthologues with new recognition capacity to overcome local microbial adaptation.

Flagellin, LPS and PGN are also classical PAMPs recognised by other higher eukaryotes; however, the actual recognised epitopes vary greatly between different kingdoms [7–9]. This clearly reflects the outcome of convergent evolution in innate immunity.

Beyond PAMPs

In addition to sensing invading microbes by means of PAMPs (infectious non-self), plants and animals can also sense infectious-self or modified-self via danger-associated molecular patterns (DAMPs) [13]. For example, plants can recognise oligo-α-galacturonides released from plant cell walls by fungal hydrolytic enzymes [14]. Furthermore, Nep1 (necrosis and ethylene-inducing peptide 1)-like proteins secreted by many pathogens are recognised by plants [15•]. This recognition results from their phytotoxic modulation of the host, and can therefore be referred to as toxin-mediated immunity. Also, recognition of the PAMP-inducible endogenous AtPep peptides was proposed to amplify PTI via a positive feedback loop, and can be seen as part of the infectious-self [16•].

Update on PAMP recognition

In recent years, the first plant PRRs have been cloned. Most identified PRRs are receptor-like kinases (RLKs) or receptor-like proteins (RLPs), except for the extracellular glucan-binding protein (GBP) that binds and hydrolyses heptaglucoside from *P. sojae* [17•]. The tomato xylanase receptors LeEiX1/2 are RLPs. Intriguingly, both GBP and LeEIX1/2 lack obvious intracellular signalling domains. Therefore, other membrane-associated proteins are probably required for signal relay.

Until recently only two *Arabidopsis* RLKs involved in PAMP perception were known; the leucine-rich repeat (LRR)-RLKs FLS2 and EFR that recognise bacterial flagellin and EF-Tu, respectively [18••,19•]. Interestingly, the *Arabidopsis* LysM-RLK CERK1/LysM-RLK1 was recently shown to be required for chitin responses [20•,11•]. However, it is not known if CERK1/LysM-RLK1 is required for chitin binding, and therefore if it is a PRR *per se*.

Notably, in rice the LysM-containing transmembrane protein CEBiP is required for chitin binding and responses [21••]. As CEBiP lacks intracellular-signalling domains, it might interact with the rice CERK1/LysM-RLK1 orthologue to transduce the chitin signal. Conversely, CEBiP orthologues might be required for chitin binding in *Arabidopsis*.

FLS2 and BAK1: update on flagellin recognition and immediate downstream signalling

FLS2 is the best studied plant PRR. AtFLS2 orthologues have been recently identified in *Nicotiana benthamiana* and tomato, based on sequence homology and requirement for flg22 perception [22•,23••]. In tomato the minimal recognised flagellin epitope is smaller and the recognised epitope more diverse than in *Arabidopsis*, demonstrating the evolutionary adaptation and flexibility of PAMP recognition [22•]. In the AtFLS2 extracellular domain the LRRs 9–15 were identified as contributing significantly to flg22 binding [24•]. However, the specific flg22-binding site could not be identified and the authors proposed that a limited number of amino acids quantitatively contributed to flg22 binding.

Flg22 binding leads to AtFLS2 endocytosis from the plasma membrane into intracellular mobile vesicles [25]. The internalisation relies on kinase activity and requires the ubiquination-related PEST motif in the AtFLS2 C-terminus [25]. AtFLS2 endocytosis is not mediated by homodimerisation as AtFLS2 does not form homodimers in protoplasts [26]. However, flg22-mediated endocytosis of FLS2 was not observed in the protoplast system [26], which raises the question of protoplasts as a suitable system for these studies. Nevertheless, flg22 binding reduces AtFLS2 membrane mobility most probably through heterodimerisation [26].

The LRR-RLK BAK1/SERK3 rapidly forms complexes with AtFLS2 upon flg22 binding and positively regulates flg22 responses [27••,28••]. BAK1 also interacts with and phosphorylates the LRR-RLK brassinosteroid hormone

receptor BRI1 [29]. Additionally, defence responses triggered by elf18 in *Arabidopsis*, or csp22 and the *Phytopthora infestans* elicitin INF1 in *N. benthamiana* are also partially compromised in plants defective in *BAK1* expression [27••,30••]. However, BAK1 is not involved in flg22 binding [27••]. Therefore BAK1 does not act as a co-receptor but rather as a signal transducer most probably relying on its kinase activity.

Interestingly, *bak1 Arabidopsis* mutants are not more susceptible to bacteria [30••]. By contrast, *N. benthamiana* silenced for *NbBAK1* are more susceptible to bacterial pathogens [28••]. The discrepancy in the requirement of BAK1 for bacterial resistance could be explained by silencing of close *BAK1* paralogues in *N. benthamiana*, which in *Arabidopsis* could partially substitute for BAK1 loss. The best candidate is BKK1, a BAK1 paralogue that acts redundantly with BAK1 in cell death control and brassinosteroid signalling in *Arabidopsis* [30••,31••]. This cell death control probably contributes to the hypersusceptibility of *bak1* mutants to necrotrophic pathogens [30••].

Overall, BAK1 appears to be a general regulator of LRR-RLKs, with newly defined roles in PTI signalling and cell death control that are independent of brassinosteroid signalling. It is probable that BAK1 interacts with and regulates the activity of yet other unknown RLKs.

PTI responses, signalling and regulation: many read-outs, little genetic proof

PAMP perception triggers many different traceable molecular, physiological and pathogenesis-related changes. Seconds to minutes after PAMP treatment there is ion-flux across the plasma membrane, increased intracellular Ca^{2+} concentration, oxidative burst, MAP kinase (MAPK) activation, protein phosphorylation, receptor endocytosis and protein–protein interaction, for example [5,32•]. By 30 min transcriptional changes are induced. They comprise up to 3% of the *Arabidopsis* genome and significantly overlap with the ETI transcriptome [18••,33]. Elf18 and flg22 induce the same set of genes, and ~30% or ~50% of those are also regulated by PGN or chitin, respectively [8,18••,11•]. This highly significant overlap in transcriptional regulation by different PAMPs clearly suggests signal convergence after PAMP recognition. Furthermore, comparison of *Arabidopsis* whole-genome transcriptional changes following infection with non-adapted, adapted and non-virulent bacteria identified over 800 PAMP-regulated genes [34,35]. Ninety-six were up-regulated over a prolonged time period of 12 hours post-infection by *Pseudomonas syringae* pv. *tomato* DC3000 mutants defective in their type-III secretion system, as well as by four different PAMPs (flg22, elf18, LPS and HrpZ). Thus, these genes could be the core primary PTI response [35]. Recently, expression profiling experiments using mini-arrays designed to analyse pathogen-responsive genes revealed the partial dependence of PTI-mediated gene induction on SA-signalling [36]. PAMPs are able to increase SA levels and mutations in the SA-signalling network abate PTI-induced defence [37,38].

Other responses that occur within hours are the enhancement of ethylene biosynthesis, stomatal closure and callose deposition [32•]. Despite the plethora of biological read-outs the signalling, regulation and interrelationship between these events and their genetic basis are only emerging slowly.

MAP kinases and WRKYs: positive and negative regulation of PTI

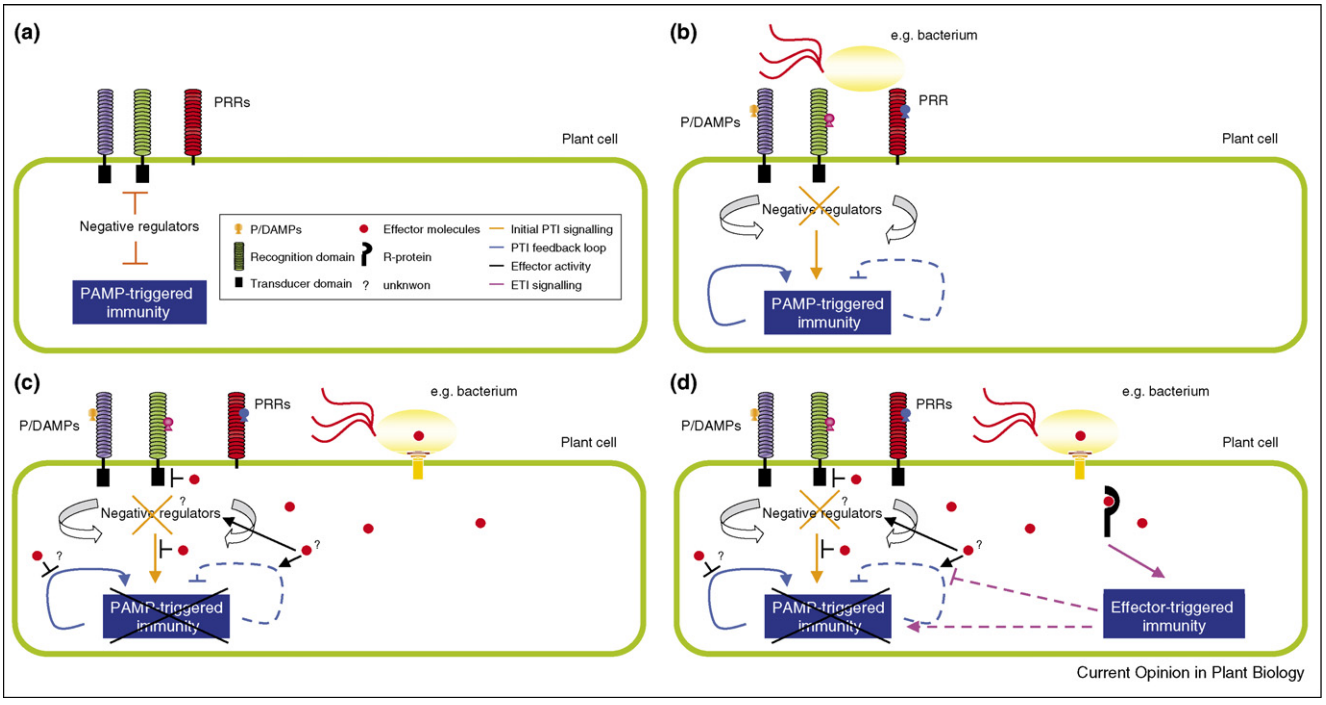
Different members of the MAPK and WRKY transcription factor families are components of downstream PTI signalling. The MAPK cascade AtMEKK1, AtMKK4/5 and AtMPK3/6 leading to the activation of WRKY22/29 was previously proposed as a positive regulatory module of flg22 signalling using constitutively active kinases in protoplasts [39]. However, recent genetic studies concluded that AtMEKK1 does not regulate AtMPK3/6 activity but rather acts upstream of the negative defence regulator AtMPK4 [40–42]. Furthermore, the positive defence regulator AtMKK1 activates both AtMPK3/6 and AtMPK4 [43]. It is therefore apparent that PTI not only up-regulates defence responses but also initiates its own negative feedback regulation (Figure 1). Together this demonstrates that interconnected MAPK networks regulate PTI signalling. Additionally, the specificity of individual MAPKs is questionable, as MPK3/6 is also involved in stomatal patterning and jasmonic acid signalling, for example [44,45].

Let us take on the negative regulators

Plants have to strike a balance between fast and efficient defence activation and penalising inappropriate expression of defence genes and uncontrolled cell death [46]. In animal innate immunity it is of paramount importance to control the strength and duration of innate immune responses to prevent over-reaction of the immune system (e.g. sepsis) and autoimmune diseases [47]. There has been much recent progress in identifying negative regulators of mammal innate immunity. These range from TAM (Tyro3/Axl/Mer) family members and Toll-like receptors, inactive signalling mimics, phosphatases, ubiquitin lyases and ligases, prolyl isomerases, transcription factors, to micro-RNAs [48,49,50•,51••,52]. Negative regulators can either be constitutively expressed or transcriptionally induced, and/or additionally regulated post-translationally, leading to a regulatory network of interconnected positive and negative feedback loops [47,51••].

Several lines of evidence suggest a similar regulatory network exists in plant defence. Firstly, treatment with the protein synthesis inhibitor cycloheximide elicits

Figure 1



Conceptual model of plant innate immunity. (**a**) Hypothetical constitutive negative regulation of PTI. Plants tightly regulate their defence responses in the absence of microbes by constitutive negative regulators. (**b**) Activation of PTI by recognition of infectious-self and non-self. Plants recognise invading microbes (e.g. bacteria) by sensing PAMPs/MAMPs or DAMPs via PRRs. This triggers diminution of the negative regulation and consequently activation of downstream signalling cascades leading to PTI. This activation is in turn dampened and/or enhanced by negative and/or positive feedback loops, respectively. (**c**) Suppression of PTI by pathogen effectors. Pathogens can suppress PTI via effector molecules. This includes binding to and modification of PRRs and signalling components. Additionally, effectors could mimic or stabilise negative regulators. (**d**) Activation of ETI by recognition of pathogen effectors. Some plants recognise individual effector molecules leading to ETI. This could be partially achieved by sequestering PTI negative regulators and possibly by reinforcing PTI-induced transcriptional changes. For definitions and identities of molecular players involved, please refer to main text.

similar gene expression to untreated seedlings following flg22 application [53], suggesting the presence of unstable negative regulators, which are rapidly degraded after defence elicitation. Secondly, three E3-ligases involved in cell death control are rapidly induced after flg22 treatment and ETI [54,55]. These are primary candidates to degrade constitutively present negative regulators. Thirdly, it became a paradigm that plant hormones de-repress signalling pathways that are normally constitutively suppressed [56].

Our knowledge about negative regulation of PTI is limited but it is highly probable that post-translational modification of host proteins are required to suppress PTI (see Block *et al.*, this issue). The kinase-associated protein phosphatase binds to AtFLS2 and abrogates downstream signalling [32•]. AtNUDT7, a nucleoside diphosphate hydrolase, negatively regulates basal defence via an unknown mechanism [57,58]. An expression profile study with four *Pseudomonas* strains carrying different effectors triggering ETI supports the idea of the importance of ADP-ribosylation in plant defence. Furthermore, NUDT7 also negatively regulates ETI, strengthening the notion of interconnected PTI and ETI signalling networks [59]. Interestingly, the *Pseudomonas* effector HopU1 ADP-ribosylates the glycine-rich RNA-binding protein GPR7 most probably interfering with RNA binding and disturbing the amount of PTI-related transcripts [60••]. Plants expressing *HopU1* or mutated in *GRP7* are indeed more susceptible to *Pseudomonas* infection.

RIN4, an interactor of the R-proteins RPM1 and RPT2, was also suggested to be a negative regulator of PTI. However, it is still an unsolved question how this proposed function can be reconciled with its degradation by the effector AvrRpt2 [61]. The *Arabidopsis* homologue of the human transcription factor NF-X1 negatively regulates toxin-mediated immunity induced by trichothecene [62]. Further, several WRKY transcription factors, such as WRKY11/17 and WRKY18/40/60 are negative regulators of basal defence and with the exception of *WRKY60* are all transcriptionally up-regulated after PAMP treatment [63•,64•,65••,66]. Intriguingly, the nuclear localisation of the activated MLA10 R-protein

leads to physical interaction with the barley orthologue of *At*WRKY18/40/60 [65••]. Therefore it is conceivable that the quantitative difference between PTI and ETI is mediated by de-repressing negative feedback regulation of PTI. This is at least partially achieved by sequestration of negative regulators of PTI (Figure 1). Thus effectors might also mimic or stabilise negative regulators to suppress PTI.

Conclusion

There has been much recent progress in understanding plant defences, and PTI is a central research focus. However, not insignificant challenges lie ahead of us. We have to identify key negative and positive PTI regulators to further elucidate the complex signalling network underlying the different layers of plant innate immunity. Furthermore, the role of small RNAs in PTI signalling and the role of PTI in symbiosis are interesting and valuable areas to investigate. Finally we have to demonstrate the transferability of our work on plant innate immunity to generate broad-spectrum resistance in crops.

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