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REVIEW PAPER

Microbial recognition and evasion of host immunity

Michiel J. C. Pel[1,2] and Corné M. J. Pieterse[1,2,*]

[1] Plant–Microbe Interactions, Department of Biology, Faculty of Science, Utrecht University, PO Box 800.56, 3508 TB Utrecht, The Netherlands
[2] Centre for BioSystems Genomics, PO Box 98, 6700 AB Wageningen, The Netherlands

* To whom correspondence should be addressed. E-mail: C.M.J.Pieterse@uu.nl

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Abstract

Plants are able to detect microbes by pattern recognition receptors in the host cells that, upon recognition of the enemy, activate effective immune responses in the invaded tissue. Recognition of microbes occurs by common conserved structures called microbe-associated molecular patterns (MAMPs). Plant pathogens and beneficial soil-borne microbes live in close contact with their host. Hence, prevention of the host's defence programme is essential for their survival. Active suppression of host defences by microbial effector proteins is a well-known strategy employed by many successful plant-associated microbes. Evasion of host immune recognition is less well studied but is emerging as another important strategy. Escape from recognition by the host's immune system can be caused by alterations in the structure of the recognized MAMPs, or by active intervention of ligand-receptor recognition. This paper reviews the structure and recognition of common MAMPs and the ways that plant-associated microbes have evolved to prevent detection by their host.

Key words: MAMP, PAMP, Pattern recognition receptors, defense signaling, disease resistance, effector, host immunity, immune evasion.

Introduction

Plants live in close contact with many different microbial organisms. Most of these micro-organisms have no direct effect on plant health or growth, but among the enormous diversity of microbes in the plant's microbiome are a large number of microbes that are either beneficial or pathogenic (Berendsen *et al.*, 2012). Beneficial associations include root-colonizing microbes, such as plant growth-promoting rhizobacteria and fungi. Because beneficial microbes are recognized as alien organisms, active interference with the plant immune system is fundamental for the establishment of an intimate mutualistic relationship with the host plant (Zamioudis and Pieterse, 2012). Microbial pathogens come in many different forms and are often of viral, fungal, bacterial, or oomycetal origin and display necrotrophic, biotrophic, or hemibiotrophic lifestyles. In total numbers, plant pathogens are relatively rare, as they require a high degree of specialization to fulfill their lifecycle on their host. Nevertheless, they form a major threat for plant survival and hence a sophisticated defence system is required to ward them off. To maximize both profitable and protective functions of the plant-associated microbes, it is important not only for plants to recognize microbes but also to be able to differentiate between the good and the bad and to respond accordingly. Conversely, it is important for pathogenic and beneficial microbes to modulate the host immune system to prevent effectual defences and to establish an intimate relationship. Here, we review the characteristics of some of the best-studied microbial signatures, their recognition by the host immune system, and the microbial strategies that have emerged to be important for the evasion of host immune recognition.

Non-self recognition: detection of microbial structures

Like animals, plants are equipped with an innate immune system that is activated after recognition of an invading organism

Abbreviations: EFR, EF-Tu receptor; GlcNAc, *N*-acetylglucosamine;MAMP, microbe-associated molecular pattern; LPS, lipopolysaccharide; MurNAc, *N*-acetylmuramic acid; PAMP, pathogen-associated molecular pattern; PGPR, plant growth-promoting rhizobacteria; PGN, peptidoglycan; PRR, pattern-recognition receptor; PTI, PAMP-triggered immunity; RLK, receptor-like kinase; RLP, receptor-like protein; TLR, Toll-like receptor.

(Nürnberger *et al.*, 2004; Akira *et al.*, 2006; Spoel and Dong, 2012). Recognition of non-self molecules is an important first step towards an effective immune response and is enabled by pattern-recognition receptors (PRRs) in the host cells. These PRRs are able to recognize microbe-associated molecular patterns (MAMPs), which are also often referred to as pathogen-associated molecular patterns (PAMPs) (Boller and Felix, 2009). The recognition of MAMPs/PAMPs by plant PRRs leads to so-called PAMP-triggered immunity (PTI), which provides a first line of defence against most of the non-adapted pathogens (Jones and Dangl, 2006). In mammals, Toll-like receptors (TLRs) are the most-studied PRRs, and so far 13 TLRs have been identified that are involved in the recognition of numerous different MAMPs. TLR4 for example, recognizes lipopolysaccharides (LPSs) from Gram-negative bacteria, while TLR9 is involved in the recognition of alien DNA (Akira *et al.*, 2006; Kawai and Akira, 2011). Examples of MAMP perception in plants are the perception of the conserved RNA-binding motif of bacterial cold-shock proteins (Felix and Boller, 2003) and the recognition of a conserved 17 amino acid sulphated domain of the Ax21 protein of *Xanthomonas* species by the rice receptor XA21 (Song *et al.*, 1995; Lee *et al.*, 2009). In plants, the best-characterized PRRs belong to the receptor-like kinases (RLKs) or the receptor-like proteins (RLPs). RLKs are membrane-spanning proteins with an extracellular ligand recognition domain and an intracellular kinase domain involved in signal transduction. RLPs show a similar structure but lack the intracellular kinase domain. The genome of *Arabidopsis thaliana* encodes over 600 RLKs, but for only a small number of them a role in MAMP recognition has been demonstrated. Some RLKs have been shown to function in other processes, such as development, but for most of these proteins, the function is still unknown (reviewed by Boller and Felix, 2009; Monaghan and Zipfel, 2012).

Even though 600 RLKs, in combination with all other proteins that might function in MAMP detection, offer a high number of putative receptors, the number of microbes that can be encountered is almost unlimited. Hence, to be able to detect as many different microbes as possible, plants need to recognize structures that are common in large groups of micro-organisms. A number of these common MAMPs that are recognized by plants have been well described and include chitin, peptidoglycans, LPSs, elongation factor Tu, and flagellin.

Chitin

Chitin is an *N*-acetylglucosamine (GlcNAc) polymer that forms an important component of the fungal cell wall (Fig. 1). It is recognized by the immune system of both plants and animals. Treatment of plants or plant cells with chitin leads to the activation of defence-related responses in both monocots and dicots (Shibuya and Minami, 2001). In rice, the plasma membrane glycoprotein chitin elicitor-binding protein (CEBiP) plays an important role in chitin recognition. Although CEBiP is a membrane-bound protein, it lacks an intracellular kinase domain, which suggests the requirement for additional proteins for chitin-induced signalling (Kaku *et al.*, 2006). The rice RLK chitin elicitor receptor kinase 1 (*Os*CERK1) is such a protein and forms chitin-induced hetero-oligomers with CEBiP (Kaku *et al.*, 2006; Shimizu *et al.*, 2010). *Os*CERK1 and CEBiP both contain extracellular lysine motif (LysM) domains that are involved in chitin binding. In *A. thaliana*, the chitin receptor CERK1 contains three LysM domains and binding of the chitin oligomer $(GlcNAc)_8$ to the second LysM domain leads to dimerization and activation of defence responses (Miya *et al.*, 2007; Wan *et al.*, 2008; Liu *et al.*, 2012). Smaller GlcNAc oligomers [$(GlcNAc)_{2–5}$] cannot bind to CERK1, or binding occurs but does not lead to dimerization and subsequent downstream responses (Zhang *et al.*, 2002; Petutschnig *et al.*, 2010; Liu *et al.*, 2012). *A. thaliana* also contains three CEBiP-like proteins. However, mutations in these genes do not affect chitin signalling, suggesting that these proteins are not involved in chitin perception. Instead, an additional LysM-containing receptor-like kinase called LYK4 seems to play a role in the elicitation of chitin responses (Wan *et al.*, 2012). However, the exact role of this receptor-like kinase in chitin recognition is still unknown.

Peptidoglycans

Besides its role in the recognition of fungal pathogens, CERK1 also has been associated with resistance against bacterial pathogens. For instance, *A. thaliana* mutant *cerk1* is enhanced susceptible to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (Gimenez-Ibanez *et al.*, 2009*a*,*b*; Willmann *et al.*, 2011). Additionally, *P. syringae* pv. *tomato* produces the protein AvrPtoB, which targets CERK1 for degradation, which leads to enhanced susceptibility of the plant (Gimenez-Ibanez *et al.*, 2009*b*). Although bacteria do not produce chitin, they do possess peptidoglycans (PGNs), which share structural similarities with chitin. Treatment of plants with PGN or muropeptides, which are small PGN fragments, results in the activation of defence responses (Erbs *et al.*, 2008; Gimenez-Ibanez *et al.*, 2009*a*; Willmann *et al.*, 2011). PGNs are building blocks of the bacterial cell wall and provide rigidity to the cell. In Gram-positive bacteria, PGN is present as a thick outer layer, and in Gram-negative bacteria, a thinner layer of PGN can be found between the two membranes. PGN consists of sugar chains that are formed by two alternating sugars, GlcNAc and *N*-acetylmuramic acid (MurNAc). These carbohydrate backbones are linked by short polypeptides, which are attached to the MurNAc lactyl group (Schleifer and Kandler, 1972) (Fig. 1). Although CERK1 is involved in PGN detection, CERK1 does not bind PGN with high affinity (Petutschnig *et al.*, 2010). In contrast, the two LysM domain-containing, membrane-bound proteins LYSM1 and LYSM3 do interact physically with PGNs. It has been suggested that, after PGN treatment, these two proteins form a receptor complex with CERK1 in a non-redundant way. This is supported by the fact that *lym1*, *lym3* and *cerk1* mutants are more susceptible to bacterial infection and that these three mutants do not show defence gene expression after PGN treatment (Willmann *et al.*, 2011). It must, however, be noted that these last findings are in contrast to earlier results that showed that the *cerk1* mutant still responded normally to PGN treatment (Gimenez-Ibanez *et al.*, 2009*a*).

Lipopolysaccharides

LPSs are glycoconjugates present in the outer membrane of Gram-negative bacteria. They contribute to the structure of the

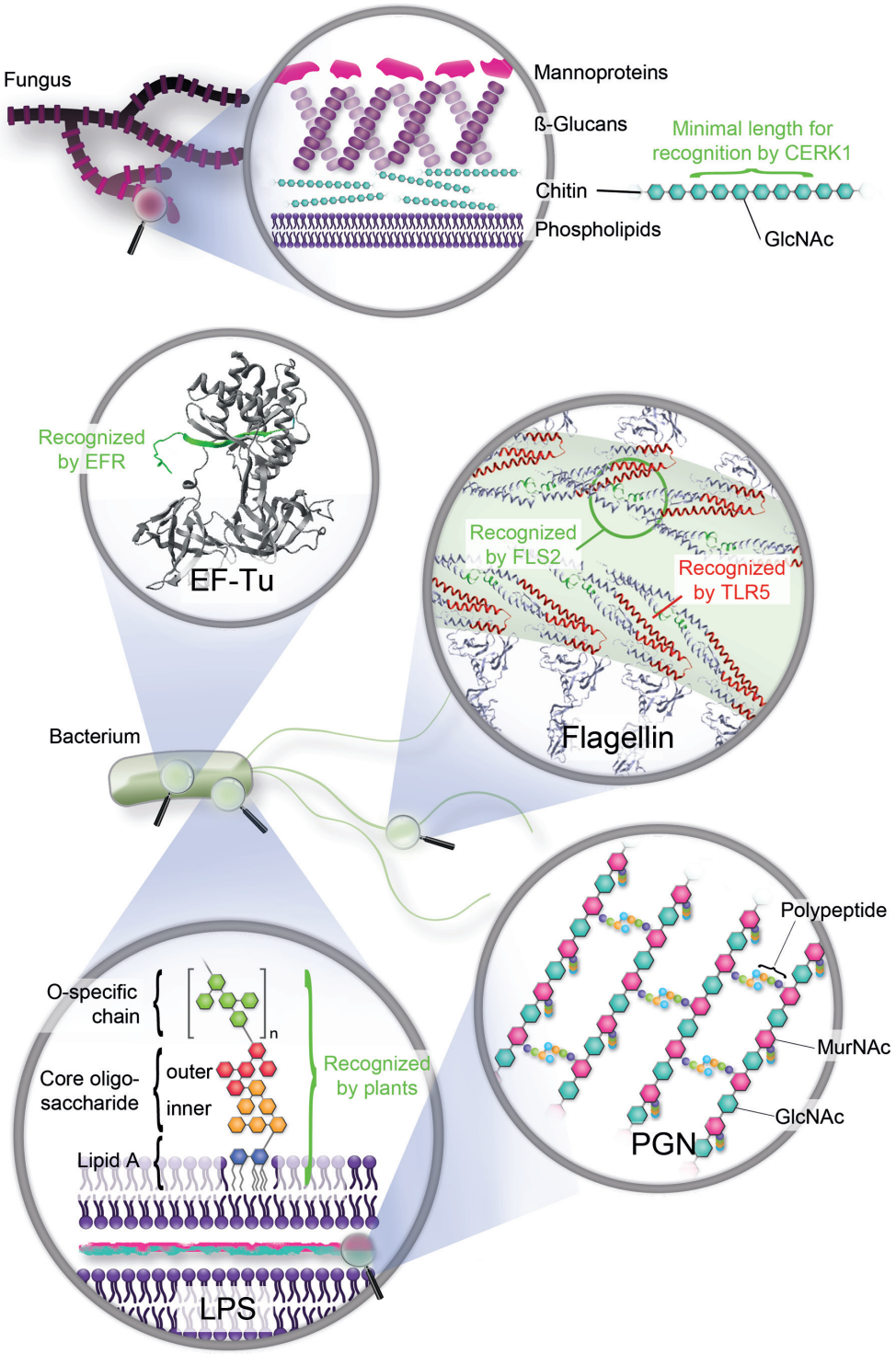

Fig. 1. Schematic representation of the structure, location and recognized domains of the described MAMPs. *Chitin:* The GlcNAc (blue) polymer chitin is an important component of the fungal cell wall. The minimal length of a GlcNAc oligomer required for dimerization of CERK1 and triggering subsequent plant immune responses is six GlcNAc molecules. *EF-Tu:* Structure of *E. coli* EF-Tu (Song *et al.*, 1999). EF-Tu is present in the bacterial cytoplasm and the acetylated N terminus of the protein (green) is recognized by the plant receptor EFR. *Flagellin:* Structure of *Salmonella typhimurium* flagellin molecules (Maki-Yonekura *et al.*, 2010). The bacterial flagella are formed by flagellin monomers. These monomers have an exposed part that forms the outside of the flagellum and a non-exposed part that is on the inside. Both the site recognized by FLS2 (green) and the site recognized by TLR5 (red) are in the conserved non-exposed part of the flagellin protein. *LPS:* LPS is anchored in the outer phospholipid layer of the outer membrane of Gram-negative bacteria. An LPS molecule generally consists of three parts: an O-specific chain (green), the core oligosaccharide (red/yellow) and the lipid A domain (blue). All three parts are able to trigger defence responses in plants. *PGN:* PGNs form the bacterial cell wall. In Gram-negative bacteria, the PGN layer is present between the two phospholipid bilayers. PGNs consist of sugar chains that contain alternating GlcNAc (blue) and MurNAc (pink). Attached to the MurNAc molecules short peptides can be found, which link the sugar backbones.

bacterial envelope and offer protection against antimicrobial compounds. LPSs generally consist of a hydrophobic lipid moiety called lipid A, an oligosaccharide core domain, and a polysaccharide called the O-specific chain or O-antigen. The lipid A domain is the most conserved domain of LPSs and consists of a phospholipid that anchors the LPS into the outer monolayer of the outer membrane of bacteria. The O-antigen of LPSs normally consists of a repeating polysaccharide with five glycosyl groups. However, this domain is highly variable, which is demonstrated by detection of over 100 different monosaccharides and more than 30 different non-carbohydrate components in this LPS domain of different species (Raetz and Whitfield, 2002; Silipo *et al.*, 2010) (Fig. 1). In addition, even more diversity is observed due to the variability in the number of polysaccharide repeats. The core domain is more conserved and can contain up to 15 monosaccharides. The inner core domain is attached to the lipid A domain and consists of a more conserved carbohydrate backbone decorated with a heterogeneous set of other residues. The outer core domain attaches the O-antigen to the LPS molecule and is more variable (Raetz, 1990; Raetz and Whitfield, 2002; Silipo *et al.*, 2010). LPSs of a wide range of bacterial species can elicit plant immune responses, such as callose deposition, nitric oxide production, production of reactive oxygen species, and increased expression of *PATHOGENESIS-RELATED* (*PR*) genes (Dow *et al.*, 2000; Gerber *et al.*, 2004; Zeidler *et al.*, 2004; Silipo *et al.*, 2005; Desender *et al.*, 2006; Silipo *et al.*, 2010). Additionally, LPSs of several bacterial species suppress the hypersensitive response or induce resistance in plants (Van Wees *et al.*, 1997; Erbs and Newman, 2003; Bakker *et al.*, 2007; Silipo *et al.*, 2010), although suppression of the hypersensitive response does not lead to increased susceptibility of the plant tissue (reviewed by Erbs and Newman, 2003).

The recognition of LPS molecules from different species suggests that plants recognize LPSs through a common conserved domain. As well as the most-conserved lipid A domain being able to trigger plant defence responses (Zeidler *et al.*, 2004; Silipo *et al.*, 2005, 2008; Madala *et al.*, 2011, 2012), the more variable core domain and O-antigen can also activate plant responses (Bedini *et al.*, 2005; Silipo *et al.*, 2005; Madala *et al.*, 2012). For many phytobacteria, the O-antigen consists of a rhamnan backbone (Molinaro *et al.*, 2009), and synthetic oligorhamnans that resemble this backbone induce defence responses in *A. thaliana*. Additionally, it has been shown that treatment with longer oligosaccharides leads to stronger activation of defence gene expression (Bedini *et al.*, 2002, 2005). Lipo-oligosaccharides (LPSs without the O-antigen) of *Xanthomonas campestris* trigger defence gene expression in *A. thaliana* in two phases, while treatment with the core domain leads to activation of the first phase and treatment with the lipid A domain triggers the second phase (Silipo *et al.*, 2005). Additionally, it has been shown that of the LPS of *Burkholderia cepacia*, the lipid A domain and the core/O-antigen domain trigger distinct gene expression patterns in *A. thaliana* (Madala *et al.*, 2012). These data suggest that the two LPS domains are differentially recognized. However, how plants recognize LPS is still unknown.

Elongation factor Tu

Another MAMP that is recognized by plants is the bacterial elongation factor Tu (EF-Tu). EF-Tu was discovered as elicitor of defence responses in 2004, and shortly thereafter, the PRR responsible for EF-Tu recognition was identified and named EF-Tu receptor (EFR) (Kunze *et al.*, 2004; Zipfel *et al.*, 2006). Comprising 5–10% of the total protein content, EF-Tu is the most abundant protein in bacteria, where it mediates the entry of aminoacyl-tRNA into the ribosome complex and in this way facilitates protein elongation (Krab and Parmeggiani, 1998). The EFR of *A. thaliana* recognizes the acetylated N terminus of ET-Tu, which leads to heteromerization of EFR with BRI1-ASSOCIATED KINASE 1 (BAK1) and the activation of downstream defence responses (Fig. 1). In addition, it has been shown that the short peptide elf18, containing the first 18 amino acids of the protein, triggers similar responses (Kunze *et al.*, 2004; Zipfel *et al.*, 2006; Segonzac and Zipfel, 2011). EF-Tu is present in the bacterial cytoplasm making it unavailable for recognition by the EFR. Probably, the high abundance of EF-Tu results in sufficient amounts of this protein for detection by the plant when bacteria die and lyse during plant infection. Additionally, there are some reports of surface-localized EF-Tu (Dallo *et al.*, 2002; Zipfel, 2008). In contrast to EF-Tu, which is widespread among bacteria, the presence of the EFR seems to be restricted to a small group of plants. This PRR has only been found in members of the Brassicaceae family, indicating that EF-Tu recognition has been acquired only recently during evolution (Kunze *et al.*, 2004). Interestingly, heterologous expression of *A. thaliana* EFR in the non-Brassicaceae plant species *Nicotiana benthamiana* and *Solanum lycopersicum* leads to the ability to recognize EF-Tu, which results in increased resistance to bacterial pathogens (Zipfel *et al.*, 2006; Lacombe *et al.*, 2010).

Flagellin

A MAMP that, in contrast to EF-Tu, is recognized by members of all groups of higher plants is the main subunit of the bacterial flagellum, named flagellin (Felix *et al.*, 1999; Boller and Felix, 2009). The flagellum enables bacterial motility and consists of an engine, a propeller, and a hook that connects the propeller to the engine. The engine consists of several proteins that reside in the cell wall and membranes, and drives flagellum rotation by an ion-powered motor. The propeller, or filament, is made entirely of flagellin proteins and can consist of up to 20 000 flagellin molecules (Samatey *et al.*, 2001; Chevance and Hughes, 2008). The perception of flagellin in plants was discovered after treating cell cultures of tomato with boiled *P. syringae* pv. *tabaci* cells. The observed defence responses were the result of the highly sensitive recognition of a conserved N-terminal domain of flagellin by the plant PRR FLAGELLIN SENSING 2 (FLS2) (Fig. 1) (Felix *et al.*, 1999; Gómez-Gómez *et al.*, 1999; Gómez-Gómez and Boller, 2000). Again, as with EF-Tu recognition, binding of the ligand to the receptor leads to heterodimerization with BAK1, which is important for downstream defence signalling (Chinchilla *et al.*, 2007; Heese *et al.*, 2007; Segonzac and Zipfel, 2011). Furthermore, it has been shown that treatment with the peptide

flg22, which contains the 22 corresponding amino acids of the conserved N-terminal domain of flagellin, also leads to strong defence activation (Felix *et al.*, 1999). Mammals are able to recognize flagellin in a similar way and this recognition by the PRR TLR5 results in host inflammatory responses. However, TLR5 recognizes a conserved domain different from the domain recognized by FLS2, suggesting independent evolutionary origins of these receptors (Hayashi *et al.*, 2001; Smith *et al.*, 2003). Even though FLS2 and TLR5 recognize different sites of the flagellin molecule, both sites can be found in the conserved flagellin domain that is hidden inside the flagellum. This raises the question of how plants and animals recognize these molecules that are not surface exposed. This is probably due to the release of monomeric flagellin molecules from dead bacteria, damaged flagellum polymers, or active shedding of flagellin molecules (Gerstel *et al.*, 2009). Again, as with EF-Tu recognition, the high abundance of the protein results in the requirement for only a small percentage of flagellin to be released for defence activation.

MAMP recognition: what defines a good MAMP?

The five MAMPs described above are very different in structure, come from different organisms, and have different functions in the micro-organisms. However, when comparing them, they have a number of characteristics in common that make them suitable ligands for plant PRRs. Firstly, they are widespread. Chitin can be found throughout the fungal kingdom, while PGN and EF-Tu can be found in all bacteria. LPS is present in all Gram-negative bacteria and flagellin is widespread among many bacterial species as well (Krab and Parmeggiani, 1998; Dow *et al.*, 2000; Yonekura *et al.*, 2002; Chevance and Hughes, 2008; Lee *et al.*, 2008; Silipo *et al.*, 2010). Secondly, they are conserved. Almost the entire EF-Tu sequence shows over 90% sequence similarity among many different bacteria (Kunze *et al.*, 2004). Additionally, even though the exposed domain of flagellin is highly variable (from almost absent up to 1000 amino acid residues), the flagellin protein is highly conserved in the non-exposed domain of the protein (Felix *et al.*, 1999; Smith *et al.*, 2003; Bardoel and Van Strijp, 2011). Furthermore, chitin and PGNs of different species are very similar, and the only observed variation can be found in the peptide chains of PGN that link the sugar backbones and the deacetylation of GlcNac in some species (Silipo *et al.*, 2010; Erbs and Newman, 2012). In contrast, LPS is highly variable compared with the other four MAMPs. However, LPSs contain a more conserved part as well, which is the lipid A domain (Silipo *et al.*, 2010). Thirdly, they are abundant. As major components of the fungal or bacterial cell wall, chitin and PGN are present at high levels and chitin is even thought to be the second most-abundant polysaccharide in the world (Lee *et al.*, 2008; Silipo *et al.*, 2010). In addition, LPS molecules are spread around the surface of bacteria, which requires high numbers of these glycoconjugates (Silipo *et al.*, 2010). Furthermore, both EF-Tu (5–10% of total bacterial protein) and flagellin (one flagellum can contain around 20 000 monomers) are present at relatively high levels (Krab and Parmeggiani, 1998; Samatey *et al.*, 2001; Chevance and Hughes, 2008). Lastly and most importantly, they are essential, which explains why they are widespread and highly conserved. As the major components of the fungal or bacterial cell wall, chitin and PGN are indispensable for the viability of these microbes (Silipo *et al.*, 2010). Additionally, EF-Tu is required for protein formation, and it has been shown that inactivation of one EF-Tu-encoding gene is only possible if a second EF-Tu-encoding gene is present (Hughes, 1990; Krab and Parmeggiani, 1998). Furthermore, the LPS lipid A domain, together with a small part of the core domain, is required for bacterial growth (Raetz and Whitfield, 2002). By contrast, the production of flagella is not essential for bacterial survival, but pathogenic bacteria that are disturbed in their flagellum production are severely affected in their virulence (Feldman *et al.*, 1998; Schmitt *et al.*, 2001).

Hence, by targeting widely distributed indispensable microbial structures for recognition, plants are able to detect a wide range of microbes. The high abundance of the MAMPs described above might help the plant to detect the presence of even small numbers of microbes, and in this way an early infection can be arrested. The recognition of conserved sites enables plants to detect large groups of microbes with only a limited number of receptors. However, for pathogen survival, recognition is not desirable, and pathogenic microbes have therefore evolved ways to circumvent MAMP detection.

Escaping recognition: MAMPs under selective pressure

The high conservation of the MAMPs that are recognized by plants is, at first glance, somewhat surprising, as their recognition should result in a selective pressure for alterations in the recognized domains. However, such alterations possibly would entail a microbial fitness penalty. Hence, there are two opposing selective forces that shape the evolution of MAMPs, and the outcome of this is different for each MAMP and species.

For mimicking EF-Tu-triggered defence responses, plants are often treated with the peptide elf18, which is derived from the *Escherichia coli* EF-Tu sequence. Although the N-terminal EF-Tu sequence is identical in many species, there are also species that show changes in this 18 amino acid-recognized domain compared with the *E. coli* sequence. Among these are the plant pathogens *Agrobacterium tumefaciens*, *P. syringae* pv. *tomato* and *Xylella fastidiosa* that show 4, 5, and 5 amino acid changes, respectively. The peptide resembling the *A. tumefaciens* N-terminal residue shows full defence-eliciting activity. However, the N-terminal peptides of *P. syringae* pv. *tomato* and *X. fastidiosa* show a strong reduction in defence activation capacity. The difference between *A. tumefaciens* and the other two pathogens is that the sequence changes in these last two microbes affect the amino acids of the peptide that are required for full defence activation. In contrast, the *A. tumefaciens* peptide shows changes only in amino acids in which substitution with an alanine does not result in diminished elicitor activity (Kunze *et al.*, 2004). Thus, if the selective pressure is strong enough, even highly conserved structures like EF-Tu can be adapted to avoid detection.

For flagellin, a similar escape of recognition has been observed in some species. The exposed domain of flagellin is variable in both sequence and length, but the non-exposed N and C termini that are important for proper flagellum formation are highly conserved (Bardoel and Van Strijp, 2011). As described above, it is this non-exposed region that is recognized by FLS2 and TLR5, and therefore a higher variability might be expected due to selective pressure. Although mutations in these domains can prevent host immune detection, they also results in the loss of bacterial motility (Smith *et al.*, 2003). Nevertheless, some pathogenic bacteria have been shown to produce flagellin molecules that are not recognized by TLR5 although they retained their motility. This is the result of additional mutations outside the recognized domain that preserve bacterial mobility (Andersen-Nissen *et al.*, 2005). Additionally, some strains of the plant pathogen *X. campestris* produce flagellin molecules that do not trigger defence responses in *A. thaliana* (Pfund *et al.*, 2004; Sun *et al.*, 2006). Furthermore, it has been demonstrated that flagellin from the symbiont *Sinorhizobium meliloti* and the pathogen *A. tumefaciens*, both of which form long-lasting associations with the plant, are also not eliciting immune responses in plants (Felix *et al.*, 1999). Hence, positive selection apparently can result in altered flagellin monomers that escape recognition.

LPSs of different bacteria can trigger different responses in plants. This is not surprising given the high variability of the exposed polysaccharide part of these structures. However, the more conserved core oligosaccharide shows variation among species as well, and this variation can result in different elicitor activity. For example, the core domain of *E. coli* and *Ralstonia solanacearum* LPS does not trigger defence gene expression, which is in contrast to the core domain of *X. campestris*, which does elicit defence responses (Silipo *et al.*, 2005; Erbs and Newman, 2012). Additionally, even though the structure of the lipid A domain is conserved within plant-associated bacteria, some variation can still be observed. In particular, within members of the Rhizobiaceae, the lipid A domain shows more structural divergence (Silipo *et al.*, 2010; Erbs and Newman, 2012). However, if and in what way these structural differences affect lipid A recognition by plant cells remains uncertain.

Evasion of recognition: going into stealth mode

Besides escaping recognition through evolutionary adaptations of their MAMPs, pathogens are also able to produce other proteins that interfere with the plant's defences. In the last decade, tremendous research efforts have focused on the identification and characterization of so-called effector proteins. Effectors are proteins that are secreted by microbes to interfere with host defence responses. Most of these effector proteins disturb downstream immune signalling after recognition of microbial molecules, resulting in enhanced susceptibility of the host tissue. Well-studied examples of effectors are the type III secreted proteins AvrPto and AvrPtoB of *P. syringae*. AvrPto blocks MAMP signalling by binding the kinase domain of the RLKs FLS2, EFR, CERK1, and BAK1, while AvrPtoB targets FLS2 for degradation (Göhre and Robatzek, 2008; Shan *et al.*, 2008; Xiang *et al.*, 2008; Gimenez-Ibanez *et al.*, 2009*b*). During the evolutionary arms race between pathogens and hosts, plants acquired resistance (R) proteins that recognize attacker-specific effectors, resulting in a secondary immune response called effector-triggered immunity. Ultimately, the final outcome of the battle, also known as the zig-zag model (Jones and Dangl, 2006), depends on the balance between the ability of the pathogen to suppress the plant's immune system and the capacity of the plant to recognize the pathogen and to activate effective defences. In recent years, a large number of excellent reviews have covered this topic (Boller and Felix, 2009; Boller and He, 2009; Büttner and He, 2009; Stassen and Van den Ackerveken, 2011; Bozkurt *et al.*, 2012). Besides effector proteins that suppress early immune responses in infected host tissue, recent studies have revealed proteins in both fungal and bacterial pathogens that prevent recognition of MAMPs and thus intervene before the microbe is recognized by the host. Additionally, some microbes can actively alter their MAMP structure and in this way diminish MAMP recognition.

One of the plant's responses to an infection by a pathogenic fungus is the production of fungal cell wall-degrading enzymes and chitinases. To protect itself against these chitinases, the fungus *Cladosporium fulvum* produces avirulence protein 4 (Avr4), which prevents the chitin in the fungal cell wall from being hydrolysed by binding to it (Van den Burg *et al.*, 2006; Van Esse *et al.*, 2007). Additionally, this fungus has been shown to produce and secrete an even more remarkable protein that can also bind chitin. This protein, called extracellular protein 6 (Ecp6) contains three LysM domains and has orthologues in many different fungal species (Bolton *et al.*, 2008). Although Ecp6 binds chitin with very high specificity, it does not have the same protective function as that shown for Avr4. However, by binding chitin, Ecp6 makes the chitin fragments that are normally recognized by the plant unavailable for the plant PRR CERK1, thereby inhibiting chitin-triggered immunity. In this way, Ecp6 enables the pathogen to avoid detection, thereby increasing its virulence (Bolton *et al.*, 2008; De Jonge *et al.*, 2010). Interestingly, Ecp6-like genes can also be found in human pathogenic fungi (Bolton *et al.*, 2008), suggesting that fungal pathogens on mammals might evade recognition in a similar way (Fig. 2A).

In bacteria, a similar evasion of recognition strategy has been observed. In a search for antagonists of TLR5 signalling in the supernatant of *Pseudomonas aeruginosa*, the type I secreted alkaline protease AprA was identified (Bardoel *et al.*, 2011). AprA is a zinc metalloprotease that belongs to the serralysin family of which many members are virulence factors in Gram-negative bacteria (Stocker *et al.*, 1995). Addition of AprA to human cells prior to flagellin treatment resulted in a weaker and, at higher concentrations, even a complete absence of flagellin-induced immune responses (Bardoel *et al.*, 2011). This reduction in flagellin responsiveness is the result of the degradation of monomeric flagellin molecules by AprA. Bacterial motility is not affected by AprA, as flagellin polymers that form the bacterial flagellum are not targeted by this protease. Additionally, mutants of *P. aeruginosa* that do not produce AprA induced the TLR5 signalling output in human cells over 100-fold compared with wild-type *P. aeruginosa*. Interestingly, AprA not only interferes with flagellin recognition by TLR5, but also prevents the recognition of flagellin (and flg22) by FLS2. This is supported

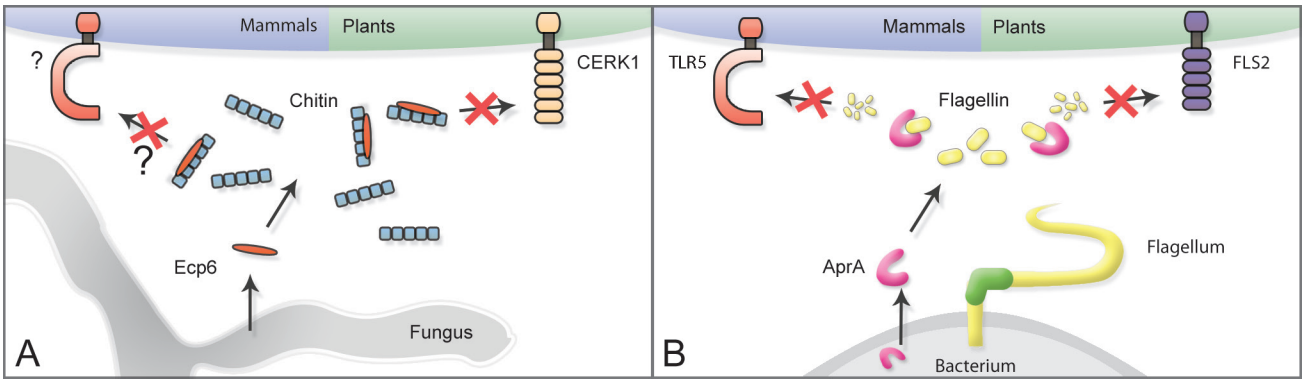

Fig. 2. Evasion of detection by fungal and bacterial pathogens. (A) Numerous fungal pathogens can produce and secrete the LysM domain-containing protein Ecp6. This protein binds the chitin molecules that are surrounding the fungus making these MAMPs unavailable for the chitin receptor CERK1 in the host cell. In this way, the fungus prevents detection of chitin molecules (De Jonge *et al.*, 2010). Whether a similar evasion of recognition occurs in mammalian hosts remains to be elucidated. (B) A number of bacterial pathogens produce the alkaline protease AprA. Upon secretion, this protease degrades the flagellin monomers surrounding the bacterium. In this way, AprA prevents detection of these MAMPs by the TLR5 receptor in mammalian cells and the FLS2 receptor in plant cells (Bardoel *et al.*, 2011).

by the fact that treatment of *A. thaliana* with *P. aeruginosa aprA* mutants resulted in faster stomatal closure compared with closure induce by wild-type bacteria. Hence, degradation of flagellin by AprA enables *P. aeruginosa* to evade recognition by the immune system of both mammals and plants (Fig. 2B) (Bardoel *et al.*, 2011). Interestingly, homologues of AprA can be found in many other pathogenic and symbiotic bacteria, which suggests that AprA is an important virulence factor.

During symbiotic interactions with plants, *Rhizobium leguminosarum* is able to alter the structure of its lipid A domain (Kannenberg and Carlson, 2001). Additionally, it has been shown that treatment of *Azospirillum brasilense* with root exudates changes the LPS profile of this bacterium (Fischer *et al.*, 2003). These alterations might increase the bacterial resistance against antimicrobial compounds or they might lead to evasion of recognition and thus a reduced PTI response in plants. *Yersinia pestis*, a parasite on humans that can be transmitted by fleas, shows such evasion of its host immune responses. *Y. pestis* produces structurally different LPS molecules at 27 °C when fleas are its host compared with at 37 °C when it is on a human host. Additionally, treatment of human cells with LPS produced by bacteria grown at 27 °C resulted in significant stronger defence activation compared with treatment with LPS produced at 37 °C (Kawahara *et al.*, 2002; Rebeil *et al.*, 2004; Erbs and Newman, 2012). The genomes of the plant pathogens *Erwinia chrysanthemi* and *Erwinia carotovora* contain homologues of the *pagP* gene that is also present in many mammalian pathogens. The *pagP* gene encodes an enzyme responsible for palmitoylation of lipid A, a process that transfers a palmitate group from a phospholipid to lipid A. Treatment of human cell lines with lipid A have revealed that palmitoylation strongly attenuates the response of these cells to lipid A (Bishop, 2005; Bishop *et al.*, 2005). LPS is normally derived from bacteria grown in culture. However, it is clear that bacteria have the ability to alter their LPS structure when environmental conditions change. For some mammalian pathogens, a role for LPS alternation in evasion of recognition has been shown, but for plant-pathogenic bacteria, data are still missing.

Another adaptation to avoid MAMP detection occurs in some *E. coli* strains. In humans, bacterial RNA can be recognized by TLR7, which leads to the activation of immune responses (Bardoel and Van Strijp, 2011). The tRNA molecules of *E. coli* are normally recognized as well. However, recently it was shown that $tRNA^{Tyr}$ of *E. coli* does not trigger TLR7 activation in human cells. This is the result of the 2′-O-methylation of residue 18 (G18) of this tRNA (Gehrig *et al.*, 2012). Moreover, tRNA of *E. coli* Nissle 1917 and *Thermus thermophilus* also contain methylated G18 (Gm18), and tRNA of both types of bacteria was shown to be non-immunostimulatory (Joeckel *et al.*, 2012). Furthermore, Gm18-modified $tRNA^{Tyr}$ reduces immunostimulation by total *E. coli* tRNA preparations (Gehrig *et al.*, 2012). This same antagonist effect was shown for the Gm18-containing tRNA molecules of *E. coli* Nissle 1917 and *T. thermophilus* (Joeckel *et al.*, 2012). Thus, as well as the presence of Gm18 in bacterial tRNA preventing immune activation by the specific tRNA molecules, Gm18-containing tRNAs also function as antagonists for TLR7. Whether bacteria can regulate this post-transcriptional modification of its tRNA in response to environmental cues remains to be elucidated.

Specific recognition: friend or foe?

In nature, plants not only interact with pathogenic micro-organisms, they also abundantly form beneficial interactions with soil-borne microbes. Classic examples of such mutualistic plant–microbe associations are mycorrhizal fungi that form a symbiosis with ~80% of all plant species and aid in the uptake of water and minerals (Van der Heijden *et al.*, 1998), *Rhizobium* bacteria that fix atmospheric nitrogen for the plant (Spaink, 2000), and plant growth-promoting rhizobacteria and fungi that stimulate plant growth and suppress plant diseases (Lugtenberg and Kamilova, 2009; Van der Ent *et al.*, 2009; Berendsen *et al.*, 2012; Zamioudis and Pieterse, 2012). Many of these microbes are present on the outside of plant roots, while others are endophytic and form a

much closer relationship with their host. As many MAMPs are widespread and conserved among microbes, beneficial microbes posses similar MAMPs as pathogens. For plants to benefit from the presence of these beneficial microbes, it is important to distinguish between pathogenic and beneficial microbes. Evidence is accumulating that suggests that beneficial micro-organisms are initially perceived as potential invaders, resulting in the activation of the plant immune system. However, like pathogens, many beneficial microbes have been shown to suppress host immunity to establish a successful relationship with their host (reviewed by Zamioudis and Pieterse, 2012). Additionally, beneficial micro-organisms also appear to have similar strategies to evade recognition.

Rhizobium bacteria form a symbiotic relationship with leguminous plants, and together they form nodules in which the bacteria fix atmospheric nitrogen. Plants recognize rhizobia initially as a threat, which leads to the elicitation of defence gene expression (Kouchi *et al.*, 2004; Lohar *et al.*, 2006; Zamioudis and Pieterse, 2012). Therefore, rhizobia need to avoid detection in a similar way to pathogens. *S. meliloti* produces flagellin molecules that do not elicit defence responses, and recently it was shown that the same is true for *Mesorhizobium loti* (Felix *et al.*, 1999; Lopez-Gomez *et al.*, 2012), supporting the importance of avoiding detection for beneficial microbes. Additionally, homologues of AprA can be found in several *Rhizobium* species. However, whether these homologues function in PTI evasion in a similar way to *P. aeruginosa* AprA is not known (Bardoel *et al.*, 2011). During the later stages of rhizobial colonization of the plant, the expression of defence-related genes is downregulated, which suggests that *Rhizobium* bacteria are able to suppress host defence responses (El Yahyaoui *et al.*, 2004; Kouchi *et al.*, 2004; Lohar *et al.*, 2006; Moreau *et al.*, 2011). One of the bacterial compounds involved in defence suppression is LPS from *S. meliloti*. Treatment of cell cultures with LPS from *S. meliloti* triggers only a weak defence response in *Medicago sativa* host plants. Furthermore, simultaneous treatment of *M. sativa* with *S. meliloti* LPS and defence elicitors from yeast results in a reduction in early and late induced defence responses. This suppressive capacity of LPS seems to be limited to the *S. meliloti–M. sativa* interaction, as non-host plants respond normally to *S. meliloti* LPS (Albus *et al.*, 2001; Scheidle *et al.*, 2005; Tellstroem *et al.*, 2007).

Plant growth-promoting rhizobacteria (PGPRs) are non-symbiotic bacteria that can stimulate plant growth (Lugtenberg and Kamilova, 2009). Like rhizobia, PGPRs trigger PTI responses in plants (Bakker *et al.*, 2007; Van Wees *et al.*, 2008). Hence, PGPRs should decrease the level of recognition by the host in order to minimize activation of host defences (Millet *et al.*, 2010). Phase variation might be a strategy for PGPRs to minimize detection when colonizing roots. Phase variation is a process in which bacteria can reversibly switch between two phenotypic stages (Davidson and Surette, 2008; Van der Woude, 2011). This phenomenon is common among rhizosphere pseudomonads and has been shown as a way for animal pathogens to evade immune detection (Van der Woude, 2011). Achouak *et al.* (2004) demonstrated that *Pseudomonas brassicacearum* shows two distinct phenotypic variants that distribute differently on plants roots. Phase I bacteria produce low amounts of flagellin and are found predominantly on the basal parts of the root. Phase II cells produce significantly higher amounts of flagellin and can be found mostly on secondary roots and root tips. Interestingly, phase I cells produce several extracellular enzymes, among which is AprA, that are not produced in phase II cells (Chabeaud *et al.*, 2001; Achouak *et al.*, 2004). The lower amount of flagellin in combination with the production of AprA in phase I cells suggests a role for phase variation in evading host immunity.

Concluding remarks

In the past decade, exciting advancements have been made in our understanding of how plants perceive microbes and how they translate this perception into an appropriate response that wards off pathogens and accommodates mutualists. In addition, a wealth of evidence is accumulating on the mechanisms by which pathogenic microbes are able to suppress or evade plant immune responses (Boller and Felix, 2009; Boller and He, 2009; Büttner and He, 2009; Stassen and Van den Ackerveken, 2011; Bozkurt *et al.*, 2012). Interestingly, beneficial microbes appear to have evolved strategies to evade host immune responses that are very similar to those discovered in pathogenic microbes. For instance, the ectomycorrhizal fungus *Laccaria bicolor* and the arbuscular mycorrhizal fungus *Glomus intraradices* were recently shown to produce symbiotic effector proteins that, like pathogen effectors, enter the host cell to suppress host immune responses and promote a symbiotic interaction (Kloppholz *et al.*, 2011; Plett *et al.*, 2011). Considering the delicate interactions between plant roots and soil-borne mutualists, many more mutualistic effectors are likely to be discovered that manipulate the host immune system to accommodate beneficial plant–microbe associations. Evidence is accumulating that the hormone-regulated plant immune signalling network is a prime target of both pathogens and mutualists (Jacobs *et al.*, 2011; Kloppholz *et al.*, 2011; Plett *et al.*, 2011; Pieterse *et al.*, 2012; Zamioudis and Pieterse, 2012). The fascinating parallels between immune evasion strategies of plant and mammalian pathogens further illustrate that this field of plant and animal biology provides conceptual benefits for both fields of research, and will enable us to develop new approaches to combat pathogenic infections and maximize profitable functions of host-associated microbes.

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