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MAMP (microbe-associated molecular pattern) triggered immunity in plants

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INTRODUCTION

Plants are sessile organisms that are under constant attack from microbes. They rely on both preformed defenses, and their innate immune system to ward off the microbial pathogens. Preformed defenses include for example the cell wall and cuticle, which act as physical barriers to microbial colonization. The plant immune system is composed of surveillance systems that perceive several general microbe elicitors, which allow plants to switch from growth and development into a defense mode, rejecting most potentially harmful microbes. The elicitors are essential structures for pathogen survival and are conserved among pathogens. The conserved microbe-specific molecules, referred to as microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs), are recognized by the plant innate immune systems pattern recognition receptors (PRRs). General elicitors like flagellin (Flg), elongation factor Tu (EF-Tu), peptidoglycan (PGN), lipopolysaccharides (LPS), Ax21 (Activator of XA21-mediated immunity in rice), fungal chitin, and β-glucans from oomycetes are recognized by plant surface localized PRRs. Several of the MAMPs and their corresponding PRRs have, in recent years, been identified. This review focuses on the current knowledge regarding important MAMPs from bacteria, fungi, and oomycetes, their structure, the plant PRRs that recognizes them, and how they induce MAMP-triggered immunity (MTI) in plants.

Keywords: innate immunity, MAMPs, Flg22, EF-Tu, Ax21, PGN, LPS, Chitin

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Plants are sessile organisms that are under constant attack from microbes. They rely on both preformed defenses, and their innate immune system to ward off the microbial pathogens. Preformed defenses include for example the cell wall and cuticle, which act as physical barriers to microbial colonization. The plant immune system is composed of surveillance systems that perceive several general microbe elicitors, which allow plants to switch from growth and development into a defense mode, rejecting most potentially harmful microbes. The elicitors are essential structures for pathogen survival and are conserved among pathogens. The conserved microbe-specific molecules, referred to as microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs), are recognized by the plant innate immune systems pattern recognition receptors (PRRs). General elicitors like flagellin (Flg), elongation factor Tu (EF-Tu), peptidoglycan (PGN), lipopolysaccharides (LPS), Ax21 (Activator of XA21-mediated immunity in rice), fungal chitin, and β-glucans from oomycetes are recognized by plant surface localized PRRs. Several of the MAMPs and their corresponding PRRs have, in recent years, been identified. This review focuses on the current knowledge regarding important MAMPs from bacteria, fungi, and oomycetes, their structure, the plant PRRs that recognizes them, and how they induce MAMP-triggered immunity (MTI) in plants.

Keywords: innate immunity, MAMPs, Flg22, EF-Tu, Ax21, PGN, LPS, Chitin
Table 1 | Microbe-associated molecular patterns (MAMPs) and Damage-associated molecular patterns (DAMPs).

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<td><strong>DAMPs</strong></td>
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<td>Systemin</td>
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wall include the deposition of the β-(1–3) linked glucan cal- lose. PR proteins comprise a number of families that include enzymes, such as β-(1–3) glucanase and chitinase, which can directly attack pathogen structures, antimicrobial peptides and small proteins, and PR1, which is of unknown function [for reviews, see Hammond-Kosack and Jones (1996); Greenberg (1997); Lamb and Dixon (1997); Nürnberger and Kemmerling (2006)]. The induction of MTI in plants has been most extensively studied using the small peptides flg22 and elf18 derived from the bacterial MAMPs flagellin (Flg) and the translation elongation factor Tu (EF-Tu), respectively (Felix and Boller, 2003a; Felix et al., 2003b). Bacterial glycoconjugates, such as the pеп- tidoglycan (PGN), which provides rigidity and structure to the cell envelopes of both Gram-negative and Gram-positive bacteria (Erbs et al., 2008a; Willmann et al., 2011), and lipopolysaccharides (LPS) from the outer membrane of Gram-negative bacteria have been found to act as elicitors of plant innate immunity (Silipo et al., 2005; Erbs and Newman, 2012). Oligosaccharides derived from cell wall polymers of fungi and oomycetes also act as MAMPs. Fungal chitin and its degradation products N-acetyl-chito-oligosaccharides, i.e., chitin oligomers induce various defense responses in both monocot and dicot plants (Kaku et al., 2006; Miya et al., 2007). In the oomycetes, the cell walls are composed of β-glucans and cellulose, rather than chitin, as in the fungi. Some of the earliest work on the role of glyco- sylated compounds in triggering plant defenses has examined the effects of β-(1→3/1→6)-linked glucans from the cell walls of Phytophthora megasperma f. sp. glycinea on the induction of phytoalexin accumulation in soybean [reviewed in Cheong and Hahn (1991)]. In the plant-virus interactions no conserved viral MAMP has been identified so far, and the primary plant defense is thought to be based mainly on RNA silencing (RNAi). By analogy with the zigzag model, viral-derived double-stranded RNA (dsRNA) is regarded as the MAMP inducing RNAi, a general plant defense mechanism or the MTI. To counteract this defense, plant viruses express RNA silencing suppressors (RSSs), many of which bind to dsRNA and attenuate RNAi (Csorba et al., 2009; Ruiz-Ferrer and Voinnet, 2009).

This review will focus on some of the important MAMPs from bacteria, fungi, and oomycetes and review the current knowledge of their structure, how they are recognized and how they induce MTI in plants. We include the slightly more unusual MAMP Ax21 from the rice pathogenic bacteria Xanthomonas oryzae pv. oryzae (Xoo). MAMPs in general activate MTI directly via their respective plant receptors, whereas Ax21 is secreted out of the bacterium via the type-I secretion system (TOSS), where it is then recognized by the rice receptor XA21, and induction of MTI follows. Finally, we will briefly describe damage-associated molecular patterns (DAMPs) of plant origin, which also induce MTI in the plant.

**BACTERIAL MAMPs**

**FLAGELLIN (Flg)**

Flagella are essential structures for the pathogenic bacteria as they provide motility and often increase adhesion of the bacteria to its host. Flg, the main building block of bacterial flagella, is well-established as a major activator of innate immunity in animals [reviewed by Ramos et al. (2004)]. Some of the first MAMPs...
recognition studies in plants were carried using Flg. Studies in mammals have shown that at least one of the conserved domains in the N-terminal and C-terminal part of the bacterial Flg, found to be involved in bacterial motility as well, is recognized by Toll-like receptor 5 (TLR5) (Hayashi et al., 2001; Smith et al., 2003). Studies in Arabidopsis, tomato, and other plants, revealed that plants respond to a highly conserved domain in the N-terminal part of the bacterial Flg, a 22 amino acid (aa) peptide, flg22 (Felix et al., 1999). In order to identify the gene involved in recognition and transduction of the flg22 elicitor signal, Gomez-Gomez and Boller (2000), used a genetic approach to screen Arabidopsis mutants after flg22 treatment and isolated several Flg sensing 2 (FLS2) mutants, which mapped to the FLS2 locus on chromosome 5. FLS2 belongs to the RLK family and has an ECD with 28 LRRs, a TM domain, and an intracellular serine/threonine kinase domain. No high-affinity binding site was found, after treatment with a radiolabeled derivative of flg22, in the Flg insensitive Arabidopsis ecotype Ws-0 and in plants carrying mutations in the LRR domain of the FLS2 gene, indicating a role for LRR in Flg binding (Gomez-Gomez and Boller, 2000; Bauer et al., 2001). Later work revealed that both an extracellular LRR domain and kinase activity of FLS2 were necessary for high affinity binding and binding specificity for Flg (Gomez-Gomez et al., 2001). Chinchilla et al. (2006) showed the specific interaction of flg22 with FLS2 in Arabidopsis. The recognized domain within Flg is not the same for all plant species. For example, flg15, an N-terminally shortened version of flg22, was shown to be highly active in tomato, while it only elicits immune responses at higher concentrations in Arabidopsis. Rice is able to recognize flg22, but its defense response is greater to the full length Flg (Takai et al., 2008). The functionality of the FLS2 receptor was tested by heterologous expression of the Arabidopsis FLS2 receptor in tomato cells. In these expression studies, tomato cells gained the Flg perception system characteristic for Arabidopsis, demonstrating that FLS2 represents the PRR that determines the specificity of Flg perception (Meindl et al., 2000; Bauer et al., 2001; Chinchilla et al., 2006). The difference in recognition of the Flg epitope is not restricted to different plant families; variations have also been found between species in the same family. A 15 aa peptide derived from E. coli Flg was shown only to be highly active in tomato (Solanum lycopersicum previously called Lycopersicon esculentum), but not in tobacco. Furthermore, the tomato Flg receptor, SlFLS2, an ortholog of the Arabidopsis FLS2 receptor, has now been identified and used in expression studies with Nicotiana benthamiana, where N. benthamiana expressing SlFLS2 gained the Flg perception system specific for tomato (Robatzek et al., 2007). In addition to this, studies focusing on host recognition of Xanthomonas campestris pv. campestris (Xcc) Flg have revealed variability within species and within pathovar families for disease eliciting activity of Flgs among Xcc strains (Sun et al., 2006). Confirmation of FLS2 as a surface receptor came with studies using transgenic Arabidopsis Ws-0, expressing FLS2 fused to the green fluorescent protein (GFP), which revealed a cell membrane localization of FLS2. Additionally, FLS2 was found to undergo ligand-induced endocytosis; it is thought that this subcellular redistribution of FLS2, or any other surface receptor, from the plasma membrane to cytoplasmic vesicles may be a central point in signaling during immune responses (McCoy et al., 2004; Robatzek et al., 2006). Flg-induced activation of FLS2 in Arabidopsis, involves a complex formation with the BRASSOSTEROID-INSENSITIVE 1 (BRI1)-associated receptor kinase 1 (BAK1) (Chinchilla et al., 2007). BAK1 has also been reported to be involved in BRI1 endocytosis (Russinova et al., 2004). Furthermore, BAK1 is required for the immune responses triggered by multiple MAMPs other than Flg, including the bacterial elongation factor EF-Tu (see below) (Roux et al., 2011). The activities of MAP kinases (MAPK) were delayed and reduced or even absent in response to flg22 or elf18, a fully active EF-Tu derivative, in bak1 mutants, compared to wild type plants. This indicates that BAK1 acts as a positive regulator of MAMP signaling in Arabidopsis. In addition, it was revealed that FLS2, after flg22 stimulation, interacts with BAK1 in a ligand-dependent manner (Chinchilla et al., 2007). This interaction allows phosphorylation and activation of the receptor complex (Sculze et al., 2010). Downstream of the FLS2-BAK1 receptor complex is a cytoplasmic receptor kinase Botrytis-induced kinase 1 (BIK1), which constitutively associate with FLS2. After FLS2-BAK1 dimerization, BIK1 dissociate from FLS2, possibly allowing BIK1 to phosphorylate downstream components, and thus linking the MAMP receptor complex to downstream intracellular signaling leading to MTI (Lu et al., 2010). However, the substrates of FLS2 and BAK1 kinases have yet to be identified, and how the MAMP signal is transmitted from the BAK1-associated receptor complexes at the plasma membrane to intracellular events is largely unknown.

ELONGATION FACTOR TU (EF-Tu)

In protein biosynthesis, the ribosomes translate the sequence of nucleotides in mRNA into the sequence of aa's in a protein. During the phase of elongation the ribosome is associated with the ribosome. One such elongation factor is EF-Tu, the most abundant protein in the bacterial cell (Jeppesen et al., 2005). The elicit activity is attributed to a highly conserved part of the N-terminus of EF-Tu, either a 26 or 18 aa peptide named elf26 or elf18. The perception of EF-Tu by the EF-Tu Receptor (EFR) is independent of Flg perception, as EF-Tu is active in plants carrying mutations in FLS2 (Kunze et al., 2004). Although many of the signaling components downstream of EFR and FLS2 are shared between them (see above), EF-Tu recognition has only been found to elicit innate immunity in members of the family Brassicaceae (Zipfel et al., 2006). Studies using crosslinking assays in Arabidopsis cells, confirmed that elf18 and flg22 bind to different high-affinity binding receptors. Nevertheless, elf18 and flg22 were found by microarray analysis to induce the same pool of genes, and also a common set of responses in Arabidopsis. In addition to this, a combined treatment with both MAMPs, elf26/18 and flg22, was shown to induce the same kinases without an additive effect (Zipfel et al., 2006). An EF-Tu insensitive efr-1 mutant did not respond with an oxidative burst, increased ethylene biosynthesis or induced resistance to Pseudomonas syringae pv. tomato (Pst) DC3000 in response to EF-Tu-derived elicitors, whereas Arabidopsis Col-0 and the fls2 mutant did respond to EF-Tu elicitors. Heterologous expression studies of EFR in N. benthamiana, a plant lacking a perception system for EF-Tu, resulted in N. benthamiana with a perception system for EF-Tu, confirming
the role for EFR as a functional receptor for EF-Tu (Zipfel et al., 2006). In addition to this, efr mutants were found to be more susceptible to Agrobacterium tumefaciens (At)-mediated transformation than wild type plants, indicating that EF-Tu recognition and the subsequent defense responses reduce Agrobacterium-mediated plant transformation (Zipfel et al., 2006).

Similar to FLS2, EFR belongs to the RLK family and has an ECD with 24 LRRs, a single TM domain and an intracellular serine/threonine kinase domain (Zipfel et al., 2006). Both FLS2 and EFR are members of the subfamily LRR-XII of RLKs (Shiu and Bleecker, 2003). Besides FLS2 and EFR from Arabidopsis, the rice pathogen recognition receptor, XA21 (see below), which confers resistance to Xoo strains is also a member of the LRR-XII subfamily (Song et al., 1995; Shiu et al., 2004; Lee et al., 2006). In contrast to FLS2, but like XA21, N-glycosylation is required for EFR functionality. Mutation of a single predicted glycosylation site compromised elf18 binding despite correct localization of the mutated protein to the plasma membrane (Häweker et al., 2010).

**PEPTIDOGLYCAN (PGN)**

PGN, a molecule never found in eukaryotes, is an essential and unique membrane envelope component of all bacteria, making it an excellent target for the eukaryotic innate immune system [reviewed by McDonald et al. (2005); Dziarski and Gupta (2006)]. PGN, which provides rigidity and structure to the cell envelope of both Gram-positive and Gram-negative bacteria, is a complex molecule consisting of numerous glycan chains that are cross-linked by oligo-peptides. These glycan chains are composed of altering N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc), with short peptides attached by an amide linkage to the lactyl group of MurNAc. Several types of PGN, classified by the nature of the third residue of the stem peptide are commonly found. Typically, this is m-diaminopimelic acid (mDAP) PGN in Gram-negative bacteria and in some Gram-positive bacilli (genus Bacillus and Clostridium), whereas most other Gram-positive bacteria have L-lysine (LYS) PGN. In a recent study in tomato Nguyen et al. (2010) showed that pre-inoculation into tomato with Staphylococcus aureus PGN reduced the growth of a subsequent bacterial infection in PGN-treated tissue. This priming of defense with a MAMP is similar to that previously described for LPS (Newman et al., 2002). Early experiments with plant cells showed that the Gram-positive human pathogen S. aureus PGN was active as an elicitor in inducing extracellular alkalization of cultured tobacco cells, while no response was observed in cultured tobacco cells, suggesting a different perception system for PGN within the Solanaceae (Felix and Boller, 2003a). PGN from both Gram-positive and Gram-negative bacteria was later found to act as elicitors of plant innate immunity in Arabidopsis (Gust et al., 2007; Erbs et al., 2008a). Gust et al. (2007) showed that it was the sugar backbone of the Gram-positive S. aureus PGN that was responsible for triggering immune responses and not the breakdown product of PGN, the muramyl dipeptide (MDP) or the muropeptide dimer, which is known to be the minimal chemical structure required for triggering the innate immune system in vertebrates and insects [reviewed by Traub et al. (2006)].

Erbs et al. (2008a), on the contrary, using PGN from two Gram-negative bacterial plant pathogens, Xcc and At found that both Xcc and At PGN and its constituents functioned as MAMPs in Arabidopsis and induced immune responses such as generation of ROS, extracellular pH increase, PRI gene expression, and callose deposition. Furthermore, they showed that the muropeptides were significantly more effective at inducing defense responses than the intact PGN molecule. These observations could be indicative of different perception systems for PGN from Gram-positive and Gram-negative bacteria or differences in structures, and therefore recognition sites, of the muropeptides of human vs. plant pathogens. So far, the full structure of PGN from a Gram-positive plant pathogen has not been elucidated. In a study from 2009, Gimenez-Ibanez et al. showed that PGN from the bacterial pathogen Pst DC3000 induced the generation of ROS in Arabidopsis cek1 (chitin elicitor receptor kinase1) mutant plants, which indicated that Pst DC3000 PGN perception is independent of CERK1. In contrast, Willmann et al. (2011) reported that two of three Arabidopsis chitin oligosaccharide elicitor-binding proteins (AtCEBiP), LYM1, and LYM3, are involved in the perception/signaling of PGN (from various sources) together with AtCERK1, indicating the presence of a two-component receptor system similar to the rice chitin receptor OsCEBiP and OsCERK1 (Shimizu et al., 2010) (also see text below). All three proteins are required for PGN perception in vivo and for resistance to bacterial pathogens. Willmann et al.’s findings also showed that AtCERK1 is involved in the perception of at least two MAMP molecules, chitin and PGN in Arabidopsis. Interestingly, Shinya et al. (2012) showed that AtCERK1 serves both for chitin and PGN signaling, but AtCERK1 seems to contribute differently to the signaling. In the case of PGN signaling, the binding proteins LYM1 and LYM3 not only bind the ligand, but also contribute to the activation of AtCERK1 and downstream signaling, similarly to the function of OsCEBiP in rice. On the other hand, in the case of chitin signaling, AtCERK1 seems to function for both ligand perception and signaling (also see text below). Structurally, CEBiP is a receptor protein that contains extracellular LysM domains that are ~40 aa in length lacking a recognizable intracellular signaling domain. The LysM domains are considered to generally mediate binding to GlcNAc-containing glycans, like chitin and the backbone of PGN [reviewed by Gust et al. (2012)]. Also in monocots two LysM-containing PRRs have been shown to recognize PGN. Liu et al. (2012) reported two homologous rice lysine-motif containing proteins, LYP4 and LYP6. Both proteins bound PGN and chitin, but not LPS in vitro. Silencing either of the two proteins impaired the PGN or chitin-induced defense responses, and compromised the resistance against Xoo or the fungal pathogen Magnaporthe oryzae. These results suggest that PGN and chitin have overlapping perception components in rice.

In mammals, the recognition of PGN is complex, e.g., different receptors are found for PGN (extracellularly) and muropeptides (intracellularly). The cytosolic protein Nod2 can recognize MDP, from both Gram-positive and Gram-negative bacteria, and a lysine-containing muramyl tripeptide, but not a DAP-containing muramyl tripeptide (Girardin et al., 2003). In contrast, Nod1 only detects DAP-containing muropeptides. For instance, the human Nod1 recognizes the DAP-containing GlcNAc and MurNAc
tripepptide (Chamaillard et al., 2003). The structure of the mam-
malian NOD proteins is similar to that of the plant R proteins,
which are intracellular receptor proteins of the NB-LRR type
(Inohara et al., 2005). In plants these proteins are involved in
the recognition of specialized pathogen effectors leading to ETI (see
above). However, in animals they seem to be involved in MAMP
recognition rather than recognition of pathogen effectors. The
future will show if a system similar to the Nod system, detecting
intracellular microbial molecules, could be a possibility in plants.

LIPOPOLYSACCHARIDES (LPS)

LPS, the major component of the outer membrane of Gram-
negative bacteria, have been shown to have multiple roles in
plant microbe interactions; it is thought to contribute to the
restrictive Gram-negative membrane permeability, allowing bac-
terial growth in unfavorable environments. LPS and its derivatives
act as MAMPs and induce innate immune responses in plants
(Newman et al., 1995; Dow et al., 2000; Bedini et al., 2005; Silipo
et al., 2005). Earlier studies in plants have shown that LPS
can prevent the HR induced by bacteria. Pre-treatment of Arabidopsis
leaves with LPS and its derivatives was found to prevent the HR
caused by strains of Pst carrying the avrRpm1 or the avrRps4
genes, a phenomenon referred to as localized induced response
(LIR; Newman et al., 2002; Silipo et al., 2005). The mechanisms
behind HR prevention are still unknown, but the effects of LPS
pre-treatment are considered to be associated with enhanced resistance of the plant tissue to pathogenic bacteria, which is
thought to occur through an LPS-dependent potentiation of
plant defense responses (Newman et al., 2002). LPS consists of
a lipid, a core oligosaccharide, and an O-polysaccharide part.
The lipid, referred to as lipid A, is embedded in the outer part of
the phospholipid bilayer in the bacterial membrane. Lipid A and the core oligosaccharide are linked, usually by the sugar
3-deoxy-d-manno-2-octulosonate (KDO). The core oligosaccha-
ride consists of a short series of sugars and ends in the O-antigen,
which is composed of repeating oligosaccharide units (Raetz and
Whitfield, 2002). The O-antigen of the LPS from many phy-
topathogenic bacteria has shown to consist of oligorhamnans
(Bedini et al., 2002).

In order to know more about the structures within LPS that
trigger immune responses in plants, synthetic O-antigen polysac-
charides, oligorhamnans of increasing chain lengths, were tested in Arabidopsis. Tri-, hexa-, and nonasaccharides were synthesized
and found to suppress the HR, as well as act as MAMPs and elicit
the induction of the PR genes PR1 and PR2 in Arabidopsis. The
efficiency of HR suppression and PR gene induction improved
with increasing chain lengths of sugars in the synthetic O-antigen.
In addition, a coiled structure was observed with the increasing
chain length, indicating a role for this structure as a MAMP
and by correlation a role for the O-antigen from phytopathogenic
bacteria in plant innate immunity (Bedini et al., 2005). Studies in
mammalian cells have shown that LPS is recognized through
their lipid A moiety and this recognition was shown to gov-
ern the interactions with the innate immune system (Loppnow
et al., 1989). In addition to this, the molecular shape of lipid
A was found to directly correlate with its activity as a conical
shape of lipid A was associated with endotoxicity and a cylindrical
shape with antagonistic activity. A net negative charge of lipid
A was found to influence its molecular conformation, and with
that, its biological activity (Schromm et al., 1998, 2000). To
study if the innate immune system from the mammalian sys-
tem has parallels in the plant system, the role and mechanisms
of action of LPS and its derivatives, the core oligosaccharide and
the lipid A moiety, in plant-bacteria interactions were investi-
gated in Arabidopsis. Initially, the complete structure of purified
Xcc lipo-oligosaccharides (LOS), LPS without the O-chain, was
determined. Xcc LOS was found to be a unique molecule with a
high negative charge density and a phosphoramidate group never
found in such molecules before (Silipo et al., 2005). Xcc LOS and
derivatives have been shown to elicit induction of the PR genes
in Arabidopsis. LOS was found to induce the defense-related PR1
and PR2 genes in two temporal phases: the core oligosaccharide
induced only the early phase and the lipid A moiety only the later
phase, which suggests that both the core oligosaccharide and the
lipid A are recognized by plant cells, e.g., both act as elicitors.
These findings support the role of Xcc lipid A and the Xcc core
oligosaccharide as MAMPs of innate immunity in plants. Silipo
et al. (2005) speculated that the different LPS fragments are rec-
ognized by different plant receptors. This elicitor activity of Xcc
lipid A correlates with earlier studies by Zeidler et al. (2004), who
showed that lipid A preparations from various bacteria induced a
rapid burst of NO production that was associated with the induc-
tion of defense-related genes in Arabidopsis. In a recent study by
Madala et al. (2011) where the structure of Burkholderia cepacia
strain ASP B 2D lipid A was determined, the role of lipid A as a
MAMP in Arabidopsis was confirmed, and it was found to induce
transcriptional changes associated with plant defense responses.
Contrary to this, studies in tobacco cells, have shown that nei-
ther the lipid A nor the O-chain of the Xcc LOS molecule could
induce the oxidative burst alone, but rather it was the inner core
part of the LOS molecule that was responsible (Braun et al., 2005).
The conflict in results could reflect the different defense responses
measured after treatment with LPS and its derivatives in different
plants.

In correlation to studies in the mammalian system, where it is
well-established that the phosphorylation pattern of lipid
A affects its biological activity [reviewed by Gutsmann et al.
(2007)], it was tested whether de-phosphorylated Xcc LOS could
be recognized in plants. After de-phosphorylation of Xcc LOS
the molecule maintained only the negative charge of the KDO
residue, and rendered the molecule unable to induce LIR, sug-
Suggesting that the charged groups present in LOS play a key role
in inducing defense responses in Arabidopsis (Silipo et al., 2005).
Furthermore, from these experiments it could be concluded that
the electrostatic interactions involving the phosphate groups seem
to have a crucial function in binding not only lipid A, but also
the core oligosaccharide, to putative receptors in plants (Silipo
et al., 2005). LPS has been found, not only to induce defense
responses, but also to prime expression of plant defense responses
upon subsequent bacterial inoculation, e.g., promote an early
triggering of the synthesis of the antimicrobial compounds fer-
uloyl tyramine (FT) and p-coumaroyl tyramine (CT) (Newman
et al., 2002, 2007; Prime-A-Plant Group, 2006). The O-antigen
part of the LPS molecule is thought to be responsible for induced
systemic resistance (ISR) in Arabidopsis. Early studies showed that LPS from the rhizobacterium Pseudomonas fluorescens, as well as the live bacteria, induced ISR in carnation and radish, whereas mutant bacteria, lacking the O-antigen side chain could not induce ISR (Leeman et al., 1995; van Loon et al., 1998). In contrast to the rhizobacteria-mediated ISR, systemic activation of defense-related responses in plants upon local necrotizing pathogen infection is referred to as systemic acquired resistance (SAR). SAR is accompanied by a systemic increase in salicylic acid (SA), and SA is required for SAR signaling (Ryals et al., 1996; Schneider et al., 1996). However, recent studies suggest that recognition of the MAMPs, LPS, or Flg, and not necrotic lesion formations contribute to the bacterial induction of SAR in Arabidopsis. Treatment of Arabidopsis with P. aeruginosa LPS, Flg or non-host bacteria were shown to be associated with accumulation of SA, expression of the PR genes and expression of the SAR marker gene Flavin-dependent monoxygenase 1 in treated as well as in distant leaves (Mishina and Zeier, 2006, 2007). The signaling cascades underlying SAR and NO production after perception of LPS by plant cells have not yet been resolved. Sun et al. (2012) investigated the biosynthetic origin of NO and the role of Non-expressor of Pathogenesis-Related Genes (NPR1) to gain insight into the mechanism involved in LPS-induced resistance of Arabidopsis. NPR1 is a key regulator of SAR, and is essential for SA signal transduction (Rockel et al., 2002). Analysis of inhibitors and mutants showed that LPS-induced NO synthesis was mainly mediated by an arginine-utilizing source of NO generation. LPS (Sigma)-activated defense responses, including callose deposition and defense-related gene expression, were found to be regulated through an NPR1-dependent pathway. In contrast, Xcc LPS can induce defense responses in pepper without triggering the oxidative burst or SA synthesis (Newman et al., 2002).

The activity of LPS in plants has mostly been described in dicots, but studies in rice cells have revealed that LPS, from various pathogenic and non-pathogenic bacteria, induce a generation of ROS and defense-related gene expression in monocots, indicating that the machinery recognizing LPS is evolutionary conserved in monocots and dicots (Desaki et al., 2006, 2012). Furthermore, the two MAMPs, LPS and chitin oligosaccharide, induced a close correlation of genes in rice cells, indicating a convergence in signaling cascades downstream of recognition. In addition, the effect of LPS from various bacteria was shown to be associated with a programmed cell death (PCD) in rice cells. In contrast, LPS has never been shown to elicit PCD in dicots (Desaki et al., 2006). The mechanism by which LPS is perceived by plants is still not understood. Recent studies with fluorescein-labeled Xcc LPS in cultured N. tabacum cells revealed that LPS was rapidly bound to the cell wall and then internalized into the cell, and eventually, LPS was found exclusively inside the vacuole. These findings suggest endocytosis, comparable to the mammalian system, of Xcc LPS in tobacco cells (Gross et al., 2005). However, no PRRs for LPS and its derivatives have been characterized in plants. In the mammalian immune system, LPS form complexes with LPS-binding proteins (LBP), and this LPS-LBP complex is recognized by the membrane-bound CD14 receptor, glycosylphosphatidyl inositol (GPI)-anchored glycoprotein (Wright et al., 1990), which again is thought to associate with TLR4-MD2 to participate in LPS-induced signaling (Jiang et al., 2000; Miyake, 2004). The concentrations of LPS required to elicit most of the effects described above are in the 5–100 μg per ml range, suggesting that plants do not have the sensitivity to LPS shown by mammalian cells, which can respond at concentrations in the pg to ng per ml range. These considerations have led to suggestions that plants possess only low affinity systems to detect LPS (Zeidler et al., 2004), although plants can detect other bacterial MAMPs such as the peptides derived from Flg and EF-Tu elongation factor at sub nM levels. One complicating factor is the aggregation of LPS molecules within the purified preparations, which may affect the ability of LPS to cross the matrix of the plant cell wall to reach presumed membrane-associated receptors (Aslam et al., 2009).

Many groups have attempted to identify plant components involved in LPS recognition and perception often with conflicting results. Livaja et al. (2008) found that in Arabidopsis cells, B. cepacia LPS induced a leucine-rich repeat RLK At5g45840 by nearly 17-fold after 30 min. Furthermore, in a proteomic analysis of the changes following perception of LPS from an endophytic strain of B. cepacia in N. tabacum BY-2 cells, 88 LPS induced/regulated proteins, and phosphoproteins were identified, many of which were found to be involved in metabolism and energy-related processes. Moreover, proteins were found that are known to be involved in protein synthesis, protein folding, vesicle trafficking, and secretion (Gerber et al., 2006, 2008). In a transcription profiling of A. thaliana cells treated with LPS from B. cepacia, Livaja et al. (2008) surprisingly did not find any genes involved in callose synthesis. Furthermore, genes involved in ROS production were found to be upregulated at a very low level by B. cepacia LPS. In addition, Livaja et al. (2008) found that B. cepacia LPS only induced the PR genes PR3 and PR4, whereas studies in B. cepacia LPS treated Arabidopsis leaves revealed induction of several PR genes (Zeidler et al., 2004). Other LPS preparations, from P. aeruginosa and Escherichia coli, respectively, induce PR1 and PR5 in Arabidopsis leaves (Mishina and Zeier, 2007). The variation in results both reflects the different plant systems (Arabidopsis cell cultures contra the whole plant) and the origin of the LPS. All the above very specific effects show the ability of particular plants to recognize structural features within LPS that are not necessarily widely conserved.

Recognition of LPS/LOS in mammals is rather complex; how complex this recognition is in plants is still not known, and the mechanism of this recognition and consequent transduction steps in plants remains obscure. Gross et al. (2005) showed that, in tobacco cells, Xcc LPS was internalized 2 h after its introduction to a cell suspension, where it co-localized with Ara6, a plant homolog of Rab5 which is known to regulate early endosomal functions in mammals. It was speculated that this endocytosis in tobacco cells was, in correlation with the mammalian system, part of a down regulation of defense responses. In a recent study by Zeidler et al. (2010) localization and mobilization of fluorescein-labeled Salmonella minnesota LPS was studied in Arabidopsis. Leaves were pressure infiltrated with fluorescein-labeled S. minnesota LPS and the mobility of LPS was studied over time by fluorescence microscopy. After 1 h a fluorescent signal was observed in the intercellular space of the infiltrated leaf. The labeled LPS were visible in the midrib of the leaves after
4 h, whereas this fluorescence had spread to the smaller leaf veins near the midrib after 6 h. After 24 h it was detectable in the lateral veins. Moreover, cross-sections of the midrib 3 h after supplementation with fluorescein-labeled LPS revealed a fluorescent signal in the xylem. Using capillary zone electrophoresis a distribution of fluorescein-labeled *S. minnesota* LPS was found in the treated as well as in systemic leaves of the plant (Zeidler et al., 2010). In contrast to the results reported by Gross et al. (2005), no intracellular accumulation of the labeled LPS was observed in *Arabidopsis*. This disparity in outcomes might again be a reflection of the use of different plants, the difference in the age of the plants used (plant cell cultures vs. seedlings vs. fully developed plants) and the different defense responses measured after treatment with LPS and its derivatives.

Alterations in lipid A or other structures within LPS are known to occur during symbiotic interactions with plants (Kanennenberg and Carlson, 2001) and in response to compounds in plant root exudates (Fischer et al., 2003) and may occur during plant pathogenesis. These alterations may serve both to increase the resistance of the bacteria against host defenses and to attenuate the activity of lipid A or LPS in triggering those defenses. Characterization of the structure and function of LOS from a non-pathogenic *Xcc* mutant strain 8530, which carries a Tn5 insertion in a gene of unknown function (Dow et al., 1995), revealed that this mutant had a truncated core region. The fact that *Xcc* strain 8530 was defective in core completion led to significant modifications in the acylation and phosphorylation patterns of its lipid A, and these changes had influence on its ability to trigger innate immune responses in *Arabidopsis* (Silipo et al., 2008). The core sugars provide protection against antimicrobial compounds and attenuate the endotoxic properties of lipid A, similar to lipid A modifications seen in mammalian pathogens (Raetz et al., 2007). These findings indicate that *Xcc* has the capacity to modify the structure of lipid A and thus reduce its activity as a MAMP in plants (Silipo et al., 2008). The acyl chains of lipid A can vary, as can the number and length of them depending on growth conditions and bacterial species. Studies in mammalian cells have shown that LPS from *Shigella flexneri* encodes a weaker TLR4-mediated response than *E. coli* LPS due to differences in the acylation status of their lipid A moieties (Rallabhandi et al., 2008).

LPS from *Halomonas magadiensis*, an extremophilic and alkaliphilic Gram-negative bacteria, isolated from a soda lake in an East African Rift Valley has been found to act as an LPS antagonist in human cells (Silipo et al., 2004). *H. magadiensis* lipid A, characterized by an unusual and very low degree of acylation, was verified to inhibit *E. coli* lipid A-induced immune responses in human cells (Ialenti et al., 2006). *E. coli* lipid A, which is an effective agonistic structure of immune responses in mammalian cells, is composed of a bis-phosphorylated hexa-acylated disaccharide backbone with an asymmetric distribution of the acyl residues. Studies have revealed that structural differences on the lipid A skeleton, for example, acylation can affect its agonist/antagonist activity (Munford and Varley, 2006). In consonant with the ability in blocking enteric LPS-induced human monocyte activation, our laboratory found that *H. magadiensis* lipid A was able to antagonize the action of *E. coli* lipid A when inducing *PR1* gene expression in *Arabidopsis*. Even though the mode of perception of LPS in plants is far less-understood than in mammals and insects, these results indicate that *Arabidopsis* is sensitive to the same structures of lipid A that determine biological activity in humans (Erbs et al., 2008).

Thus far, LPS preparations used for the analysis of plant responses and for structural studies have been derived from bacteria grown in culture. We know nothing about the alterations in LPS that might occur when bacteria are within plants, although this may be highly relevant for recognition and signaling. Changes could occur in both the size distribution of LPS (alteration in the ratio of LOS to LPS) and/or in decoration of LPS with saccharide, fatty acid, phosphate, or other constituents. Increases in the sensitivity of mass spectrometric methodologies may allow development of micro-methods to analyse such changes in bacteria isolated from plants. Transcriptome or proteome profiling of bacteria isolated from plants may also give clues as to possible LPS modifications.

**ACTIVATOR OF XA21-MEDIATED IMMUNITY (Ax21)**

Even though the rice receptor XA21 has been known for a long time, the corresponding ligand Ax21 (previously known as avrXa21) was identified only recently (Lee et al., 2009). The conservation of XA21 in all sequenced *Xanthomonas* spp., *Xylella fastidiosa*, and the human pathogen *Stenotrophomonas maltophilia* suggests that it plays a key role in a biological function. Ax21 encodes a 194 aa protein (Bogdanove et al., 2011). The minimal recognized epitope mimicking Ax21 activity is a 17 aa sulfated peptide, called axY*22*, which has been shown to be 100% identical among six different *Xanthomonas* spp. (Lee et al., 2009). XA21, together with FLS2 and EFR, among the best studied PRRs, they all belong to subfamily LRR XII of the non-RD class of receptor kinases (Shiu and Bleecker, 2003; Shiu et al., 2004; Dardick and Ronald, 2006). **Xa21** was originally identified as a dominant-resistant locus conferring resistance to multiple *Xoo* races in the wild rice species *O. longistaminata* (Kush et al., 1990). Xa21 maps to chromosome 11, and already upon its discovery it was speculated to encode a gene product recognizing a determinant present in all *Xoo* races (Ronald et al., 1992). Later, the resistance of locus Xa21 was linked to a single gene, also named Xa21, encoding a receptor kinase-like protein with predicted LRR, TM, juxtamembrane (JM), and intracellular kinase domains and that
this single gene was sufficient to confer resistance to a number of \textit{Xoo} isolates (Song et al., 1995; Wang et al., 1996). \textit{Xa21} is a member of a gene family with at least seven members in rice. The closest relative to \textit{Xa21} is \textit{Xa21D} and the spectrum of resistance is identical between the two genes, but the level of resistance differs as \textit{Xa21D} only confers partial resistance. The LRR domains of \textit{Xa21} and \textit{Xa21D} are more than 99% identical, but \textit{Xa21D} lacks the TM and kinase domains and it may therefore have an extracellular function, but the mode of action is unknown (Song et al., 1997; Wang et al., 1998).

Several proteins have been shown to interact with \textit{Xa21}. The ATPase XB24 (\textit{Xa21}-binding protein 24) promotes autophosphorylation of \textit{Xa21}, which is thereby kept in an inactive state. When \textit{Axa21} binds to \textit{Xa21}, the XB24/XA21 protein complex probably dissociates, and \textit{Xa21} is activated (Chen et al., 2010). After activation, the phosphatase XB15 (\textit{Xa21}-binding protein 15) dephosphorylates \textit{Xa21} in order to deactivate it again (Park et al., 2008). A recent study has shown that upon \textit{Axa21} recognitions by \textit{Xa21}, the intercellular kinase domain is released and translocated to the cell nucleus, a translocation that is necessary for the \textit{Xa21}-mediated immune response (Park and Ronald, 2012). The rice transcription factor OsWKKY62 (also called XB10), which has previously been shown to be a negative regulator of \textit{Xa21} activity, is needed for this translocation (Peng et al., 2008; Park and Ronald, 2012). The E3 ubiquitin kinase XB3 (\textit{Xa21}-binding protein 3) is important for \textit{Xa21}-mediated resistance, as rice lines silenced in XB3 both have a decreased level of \textit{Xa21} and display reduced resistance to \textit{Xoo} (Wang et al., 2006). Less thoroughly studied are the genes \textit{Rax1}, 2, and 3 (Regulator of \textit{Xa21}-mediated immunity 1, 2, and 3 encoding a thiamine phosphokinase, a NOL1/NOL2/sun gene family member and a nuclear migration protein, respectively), which have also been shown to affect \textit{Xoo} resistance in \textit{Xa21}-containing rice plants (Lee et al., 2011).

Interestingly, the \textit{Arabidopsis} FLS2 and EFR receptors bind the artificial \textit{Axa21} derived peptide \textit{axY}22-A1, this binding trigger responses similar to the ones triggered by Flg. \textit{axY}22-A1 is identical to \textit{axY}22, except that the first aa in the peptide has been changed from Ala to Glu (Danna et al., 2011). It was previously thought that FLS2 was specific to Flg. Even though the authors analyzed their \textit{axY}22-A1 peptide stocks for the presence of Flg22 by mass spectrometry, a question was later raised whether the observations were caused by Flg22 contamination (Danna et al., 2011; Mueller et al., 2012). Mueller et al. (2012) had observed incidences of commercially produced peptides contaminated with flg22 and even minute amounts (in the range of 1 ppm) will activate FLS2 responses. Furthermore, \textit{Arabidopsis} cell cultures did not respond to treatment with \textit{axY}22 in their laboratory, therefore they concluded that the FLS2 binding observed by Danna et al. (2011) could be caused by contamination (Mueller et al., 2012). These concerns were dismissed by Danna et al. (2012), who believe that the difference in peptides used (\textit{axY}22-A1 vs. \textit{axY}22) and the differences in their experimental set-ups, could explain different results.

\textit{Axa21} is secreted from the bacterial cell through the TOSS—a fact that has been known longer than the identity of \textit{Axa21} (da Silva et al., 2004). TOSS is a relatively simple system consisting of only three protein subunits: a membrane fusion protein (MFP), an adenosine triphosphate-binding cassette (ABC) transporter, and an outer membrane protein (OMP). Three \textit{Xoo} genes with homology to the TOSS components have been shown to be required for \textit{Axa21} activity (the so called \textit{rax} genes). The genes \textit{raxA}, \textit{raxB}, and \textit{raxC} are identified as coding for a MFP, an ABC transporter, and an OMP, respectively (da Silva et al., 2004). \textit{raxA} and \textit{raxB} are arranged in a putative operon (called \textit{raxSTAB}) with the gene \textit{raxST}, which is not a part of the TOSS (da Silva et al., 2004). Instead \textit{raxST} is a sulfotransferase (Shuquo et al., 2012), which catalyze the transfer of sulfate from PAPS (3′-phosphoadenosine 5′-phosphosulfate) to a tyrosine residue. Sulfation of secreted peptides is often important for their biological function. This is also the case for the \textit{Axa21}-derived peptide \textit{axY}22, as a non-sulfated version of the peptide, \textit{axY}22 is not recognized by \textit{Xa21} (Lee et al., 2009). Based on genetic similarity and complementation studies, the two genes \textit{raxP} and \textit{raxQ} have been suggested to be responsible for the PAPS synthesis as they encode proteins with ATP sulfurylase and APS kinase activities (Shen et al., 2002). Downstream of \textit{raxSTAB} another putative operon, comprised of the two genes \textit{raxR} and \textit{raxH}, has been found. \textit{Xoo} strains mutated in these two genes do not express full \textit{Axa21} activity. These two genes are probably encoding proteins involved in a bacterial two component regulatory system (a response regulator and a histidine protein kinase), and they could be involved in the regulation of a number of genes (Burdman et al., 2004). The two component system composed of \textit{RaxR} and \textit{RaxH} have also been found to regulate the expression of another two component system composed of PhoP and PhoQ. The PhoPQ system also seems to control the TTSS, important for delivering bacterial effector molecules to the host cell, through regulation of the \textit{hrpG} gene (Lee et al., 2008).

As \textit{Axa21} was shown to be a secreted molecule (Lee et al., 2009) and the finding that the expression of \textit{raxST}, \textit{raxP}, \textit{raxR}, and \textit{raxC} are density-dependent it was suggested that \textit{Axa21} is a quorum sensing (QS) molecule (Lee et al., 2006). This was supported by a finding in \textit{S. maltophila}, showing that mutants lacking \textit{Axa21} display reduced motility and biofilm formation. Also in this organism it appears that \textit{RaxH} and \textit{RaxR} are part of a two-component system (McCarthy et al., 2011). Han et al. (2011) published the evidence for this hypothesis, thereby making \textit{Axa21} the first QS factor also functioning as a MAMP. But unfortunately critical errors of a central \textit{Xoo} strain used in the study has been found and the authors of the original paper are now in the process of repeating the experiments with a new validated strain in order to confirm the results (Comment on the PLoS homepage since January 2013). The outcome of these experiments should be followed with great interest. Knowledge of detection of small proteins, like QS in rice and other species, can be used to develop reagents to disrupt QS-mediated virulence activities.

**Fungal and Oomycete MAMPs**

**Chitin and β-glucan**

Examples of MAMPs from fungi and oomycetes include the fungal chitin and β-glucan from \textit{P. megasperma}. However, data describing how these MAMPs are recognized and how the
In tomato a *Verticillium* resistance locus *Ve* was identified that mediates resistance against race 1 strains of *Verticillium dahlia* and *V. albo-atrum*, respectively (Kawchuk et al., 2001). The characterization of the *Ve* locus revealed two genes *Ve1* and *Ve2* that encode cell-surface receptors belonging to the LRR class of RLP. Only *Ve1* was found to confer resistance in tomato. Moreover, tomato plants silenced in *BAK1*, showed higher susceptibility to infection with *Verticillium* indicating that *BAK1* is involved in Ve1-induced defense responses in tomato (Fradin et al., 2009). A putative ligand for the LRR-RLP Ve1 is the Ave1 (avirulence on Ve1 tomato) peptide. Ave1, a conserved peptide identified in several fungi and in the plant pathogenic bacteria *Xanthomonas axonopodis pv. citri*, has been found to have homology to plant natriuretic peptides (PNPs). PNPs are extracellular signaling molecules that have been shown to have a role in regulation of homeostasis under several stress conditions (Wang et al., 2011). Ave1 acts as an elicitor of disease resistance mediated by the LRR-RLP Ve1 in tomato (de Jonge et al., 2012), but a direct binding between Ave1 and Ve1 remains to be shown. Ve1 has been referred to as a PRR or an R protein accompanied by speculations that the Ave1 peptide could be an effector acting as a MAMP (Thomma et al., 2011). Future results will reveal, if it is possible to differentiate as strictly, as we do today, between MAMPs and effectors, as between PRRs and R proteins.

Two other PRRs in tomato, the LRR-RLPs Sleix1 and Sleix2, which have been shown to have homology to the tomato Ve and Cf PRRs, recognize the fungal ethylene-inducing xylanase (EIX) (Ron and Avni, 2004). EIX is a 22-kD fungal protein (β-1,4-endoxylanase) from *Trichoderma viride* that independent of its endoxylanase activity can act as an elicitor of defense responses in tomato and tobacco plants (Furman-Matarasso et al., 1999; Ron and Avni, 2004). The aa sequence of Sleix1 and Sleix2 are 84.1% identical, Sleix1 and Sleix2 both bind EIX, but their functions differ. The Sleix2 receptor has been shown to be internalized upon EIX application (Bar and Avni, 2009) and only Sleix2 transmits the signal mediated by EIX leading to plant immune responses (Ron and Avni, 2004). Sleix1, on the other hand, block the EIX signaling and the authors suggested that Sleix1 functions in inhibition of plant defense signaling and plant cell death in response to EIX (Bar et al., 2010). Using BAK1-silenced tobacco plants Bar et al. (2010) further showed that BAK1 was required for this inhibitory activity of Sleix1 on Sleix2 signaling and endocytosis.

### DAMAGE-ASSOCIATED MOLECULAR PATTERNS (DAMPs)

The plant defense system is not only recognizing microbial elicitors, some plant-derived molecules also induce plant defense responses. This sensing of infectious-self or modified-self is mediated by DAMPs (Seong and Matzinger, 2004; Boller and Felix, 2009), also referred to as microbe-induced molecular patterns (MIMPs, Mackey and McFall, 2006). Similarly the mammalian immune system detects "danger" through a series of DAMPs, now also in in this system named damage-associated. The mammalian DAMPs are derived from other tissues activating intracellular cascades that lead to an inflammatory response (Lotze et al., 2007).

In Plants the 18 aa tomato peptide systemin is an endogenous elicitor of plant defense (Pearce et al., 1991; McGurl et al., 1992). The systemin precursor prosystemin is a cytoplasmic protein and upon cell damage the released systemin acts as a DAMP on surrounding cells (Narváez-Vásquez and Ryan, 2004). Early reports showed that the RLK SR160 (the tomato ortholog of BRI1) was the receptor for systemin (Sheer and Ryan, 1999, 2002), but later reports found that null mutants were sensitive to systemin.
(Holton et al., 2007; Lanfermeijer et al., 2008). Also in Arabidopsis
a system with a putative cysteolic peptide (Pepl) activates tran-
scription of defense-related genes and induces alakization in cell
cultures. The 23 aa Pepl and the seven homologous in Arabidopsis
(Pep 1–7) are derived from the C-terminal part of their precursor
proteins PROPEP1–7 (Huffaker et al., 2006). The Pep1 receptor,
PEPR1, was found to be a LRR receptor belonging to the LRR XI
subfamily (Yamaguchi et al., 2006). Based on sequence simila-
ty a second receptor of Pep-peptides, PEPR2, has been identified.
Transcription of both PEPR1 and 2 is activated by wounding,
Methyl-Jasmonate (MeJA), Pep-peptides, and specific MAMPs.
A level of redundancy is found regarding ligand specificity of PEPR1
and 2 as they both bind Pep1 and 2, and in addition PEPR1 binds
Pep3–6 (Yamaguchi et al., 2010).

Oligogalacturonides (OG) and cutin released from plant cell
walls also function as DAMPs (Schweizer et al., 1996; Denoux
et al., 2008). Using a domain swap approach Brutus et al. (2010)
proved that WAK1 (Wall-Associated Kinase 1) function as an OG
receptor whereas a receptor for cutin still remains to be found.

CONCLUDING REMARKS

Even though MAMPs are much conserved, they are under selec-
tive pressure in adapted pathogens to evade recognition. For
example in the case of bacterial Flg, a potent inducer of MTI
in most plants, mutations in key residues of the flg22 epitope
that abolish recognition by the receptor FLS2 have been selected
for in several plant pathogens and symbionts (Boller and Felix,
2009; Lopez-Gomez et al., 2012). Notably, this positive selec-
tion seems to be more rapid than previously thought, as modern
natural isolates of Pst adapt to their tomato host through non-
synonymous mutations in the Flg-encoding gene flIC (Cai et al.,
2011). The best example so far of a glycosylated MAMP not being
recognized in plants, is the LPS molecule from the nitrogen-
fixing soil bacterium Bradyrhizobium sp. BTAi1, this LPS does
not trigger the innate immune response in different plant fam-
ilies. Aescynomene indica (the natural host of Bradyrhizobium),
Lotus japonicus, and Arabidopsis were tested for perception of
Bradyrhizobium LPS. Defense responses were not induced in
any of the tested plants. The authors determined the structure of
Bradyrhizobium LPS and found a unique LPS with an, in
nature, unprecedented chemical structure of the monosaccha-
rage forming the polymer, this “different” structure probably
prevents recognition by the LPS receptor complex in plants
(Silipo et al., 2011). MAMPs are necessary for microbial life and
therefore under strong negative selection, but their immuno-
genic epitopes are under positive selection to evade host immune
detection. These opposing evolutionary forces were recently
used to identify novel candidate MAMPs from Pseudomonas
and Xanthomonas species through an innovative bioinformatics
approach. Identifying new MAMPs may prove to be a source of
new antimicrobial agents (McCann et al., 2012).

Although plant receptors for bacterial PGN and the pro-
teaceous MAMPs Flg and EF-Tu elongation factor have been
identified, those involved in perception of LPS remain obscure.
In conclusion we expect that in the next few years we will see
a substantial increase in our understanding of the processes of
MAMPs perception and signal transduction for the deployment of
cross disciplinary approaches and ever expanding ranges of molecular experimental tools. Despite their critical
role in immunity, we know remarkably little about the range
and diversity of MAMPs. Most studies have focused on a limited
number of MAMPs as described in this review. The identifica-
tion of new MAMPs will give insight into the molecular and
evolutionary mechanisms underlying pathogen-host interactions,
and greater understanding of the mechanisms by which MAMPs
elicit defense responses may have considerable impact on the
improvement of plant health and disease resistance.

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