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Plants and animals: a different taste for microbes?

Cyril Zipfel* and Georg Felix

Plants and animals can recognize potential pathogens by detecting pathogen-associated molecular patterns (PAMPs). Significant advances over the past few years have begun to unveil the molecular basis of PAMP perception by pattern recognition receptors (PRRs). Although these discoveries highlight common recognition strategies among higher eukaryotes, they also show differences with respect to the nature of the receptors involved and the exact molecular patterns recognized. This suggests a convergent evolution of microbe sensing by the innate immune systems of these various organisms.

Addresses
Botanical Institute, Zurich-Basel Plant Science Centre, University of Basel, CH-4056 Basel, Switzerland
*Present address: The Sainsbury Laboratory, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK

Corresponding author: Felix, Georg (georg.felix@unibas.ch)

Current Opinion in Plant Biology 2005, **8**:353–360

This review comes from a themed issue on
Biotic interactions
Edited by Paul Schulze-Lefert and Edward Farmer

Available online 25th May 2005

1369-5266/$ – see front matter

DOI 10.1016/j.pbi.2005.05.004

Introduction

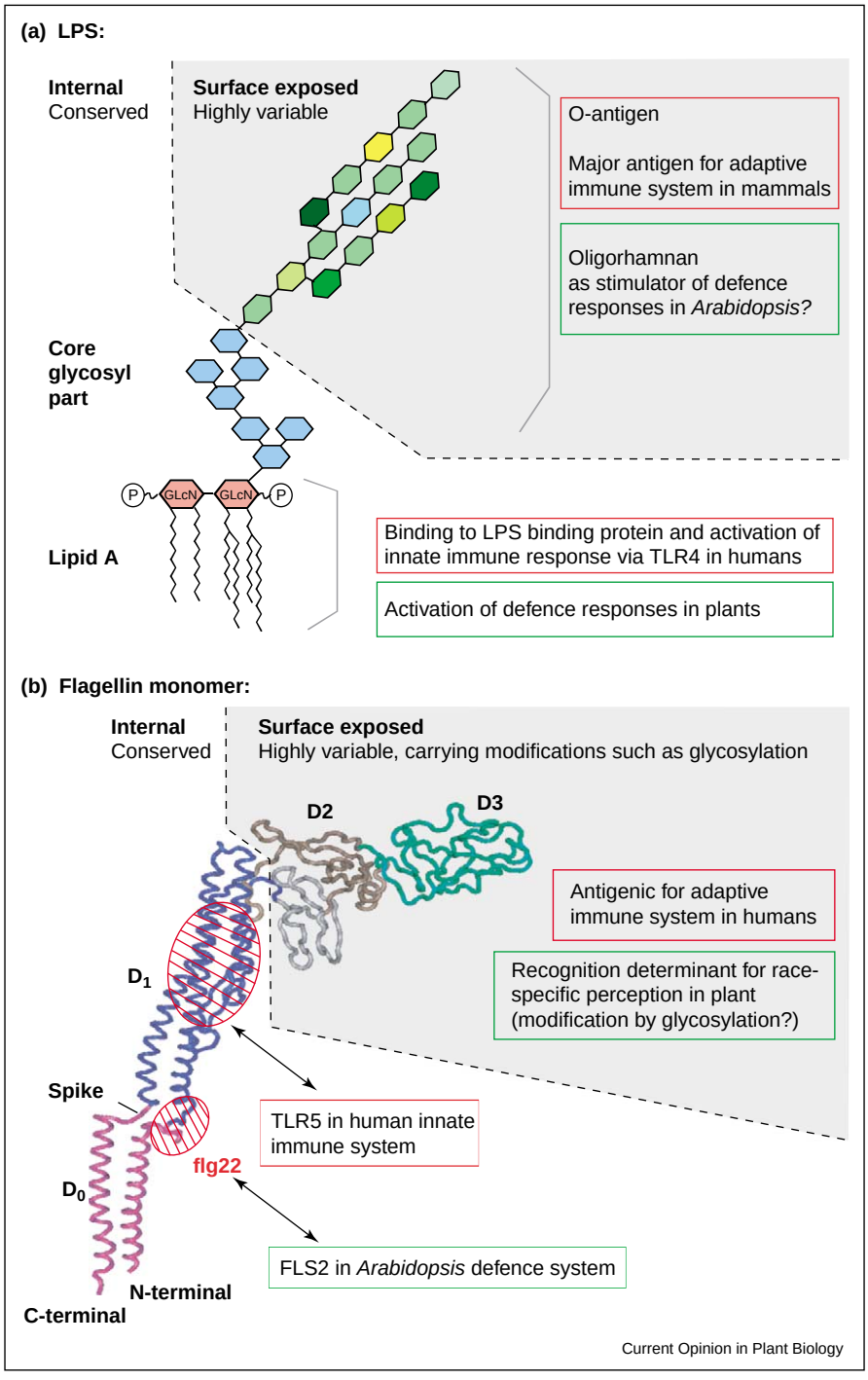
A key aspect of active defence mechanisms is a prompt and efficient detection of microbial invaders. In higher eukaryotes, this is achieved by pattern recognition receptors (PRRs), either directly by detecting pathogen-associated molecular pattern (PAMPs) [1] or indirectly by sensing wound- and injury-related structures that signal danger [2]. With the exception of antiviral RNA silencing, which exhibits features of adaptive recognition [3], plants seem to rely on 'innate' mechanisms for their defence against pathogens. The cases of plant innate immunity that have been studied most thoroughly involve a 'gene-for-gene' interaction in which dominant resistance (*R*) genes in the host plant are responsible for the recognition of pathogen-derived signals that are encoded by the corresponding avirulence (*Avr*) genes in the pathogens [4,5•]. In addition to recognition of these Avr-products, plants have perception systems for patterns that are characteristic of entire groups or classes of microorganisms, so-called general elicitors. It is now clear that general elicitors are conceptually equivalent to PAMPs [6•]. In this review, with a special emphasis on some classical bacterial PAMPs, we compare the pattern-perception systems of plants with those of insects and mammals. In our opinion, these comparisons indicate that plants, insects and mammals share common strategies of recognition but that the molecular patterns that are recognized and the receptor molecules involved differ among these three groups.

Conserved inner structure and variable surface: prototypic bacterial PAMPs

Lipopolysaccharide (LPS) is the principal component of the outer membrane of Gram-negative bacteria. It contains a long-chain polysaccharide, which is highly variable with respect to composition, length and the branching of its carbohydrate subunits (Figure 1a). This variable part of LPS, termed the O-antigen, is responsible for the enormous inter- and even intra-strain diversity of bacterial surfaces. It acts as a strong antigen for the antibody-based adaptive immune system of vertebrate animals. By contrast, the oligosaccharide core and the lipid A, which form the sheet of the membrane, are highly conserved in different bacteria. This invariable part of the outer membrane of Gram-negative bacteria is the most potent stimulator of innate immunity in mammals and is considered as a prototypic model PAMP [7•]. Several reports suggest that LPS also acts as a PAMP in plants [8••,9,10]. However, doses more than 1000-fold greater than those that induce defence responses in animals are commonly required to induce responses in plants, leaving concerns about the potential presence of minor, highly-active contaminants in the LPS preparations used. Contamination with a highly active peptidoglycan (PGN) has recently been proposed to explain earlier reports of the effects of LPS-preparations in *Drosophila* [11••]. According to this report, *Drosophila* has no PRR that recognises LPS. Support for LPS recognition in *Arabidopsis*, however, originates from the finding that the lipid A part of LPS is as effective as intact LPS in inducing a defence response [8••]. An interesting recent report also shows that synthetic oligorhamnans, which are common components of the otherwise highly variable O-chain in LPS, can trigger defence responses in *Arabidopsis* [12••], indicating that this plant species might have more than one perception system for LPS.

Flagellum-based motility is important for the virulence of bacterial pathogens [13•]. Flagellin is a protein subunit that builds up the flagellar filament. The terminal regions of this polypeptide are embedded in the flagellum inner core and build the filament architecture. The central part

Figure 1



Structure of lipopolysaccharide and flagellin. **(a)** Schematic representation of bacterial lipopolysaccharide (LPS), which forms the sheet of the outer membrane in Gram-negative bacteria. Boxes on the right indicate recognition systems for LPS that have been found in mammals [7•] and plants [8••,9,10,12••]. **(b)** Structure of a flagellin monomer from *Salmonella typhimurium* [14], based on structural data deposited in PubMed (MMDB: 24173 and PDB: 1UCU) and visualized by the CN3D program (version 4.1). Red-shaded areas and boxes indicate domains for which recognition systems have been described in mammals [13•,20••,21] and plants [15–19,22].

of the polypeptide, which is highly variable in sequence and length (Figure 1b), forms the surface of the flagellum [14]. These hypervariable surface are prime antigens for the adaptive immune system of mammals and, as recent work suggests, might also allow some plants to recognize specific strains of pathogenic bacteria [15–19]. In these

cases, glycosylation (an uncommon modification of prokaryotic proteins) of flagellin seems to allow or prevent recognition by the plant defence system. By contrast, the conserved part of the flagellin polypeptide, which faces the inside of the flagellar tube, is recognized as PAMP by the innate immune systems of plants and animals. Interestingly, mammals detect a specific part of flagellin domain D1 [13•,20••,21], whereas many plant species recognize the flg22-domain [22], which spans a part of the flagellin polypeptide termed 'spike' (Figure 1b).

Peptidoglycans build the cell wall of Gram-positive bacteria and are also present as a thin layer in the periplasmic space of Gram-negative bacteria. PGN is formed by polymer strands of muramyl dipeptide (MDP) that are cross-linked by short peptides that can vary considerably between different bacterial strains. In *Drosophila*, PRRs for PGN-fragments discriminate Gram-positive and Gram-negative bacteria and trigger different sets of defence responses [11••]. Mammals, but not *Drosophila*, are responsive to the minimal motif MDP [11••,23], indicating that insects and mammals have evolved different perception systems for this complex bacterial structure. In plants, a single report suggests that PGN might be active as an elicitor of defence responses [24]. This activity was not characterized in detail, however, and in analogy to the problem of contaminants discussed above, might be due to a minor highly active component present in the PGN preparation used. Indeed, the most-active component identified in a crude PGN preparation from *Staphylococcus aureus* was a bacterial cold-shock protein (CSP) [24]. Despite their name, CSP proteins are universal constitutive bacterial proteins. The epitope that is active as a PAMP in tobacco and other *Solanaceae* is the RNA-binding motif that is conserved in all of these proteins.

Arabidopsis and other *Brassicaceae*, by contrast, have a perception system for the amino-terminus of elongation factor Tu (EF-Tu), the most abundant protein in the bacterial cytoplasm [25•]. EF-Tu is essential for protein translation and is one of the most-conserved proteins known in bacteria.

As illustrated above, molecular structures that are essential for the architecture and function of microbial cells are often not freely exposed to the cell surface. Nevertheless, plants and animals have evolved systems that are able to recognise such hidden or embedded structures, and perception systems also exist for structures that are known to reside within the cytoplasm of the microbes. Examples include the detection of EF-Tu [25•] and CSP [24] by plants and the perception of heat shock proteins and non-methylated bacterial DNA by mammals [26]. While greatly extending the repertoire of structures that might serve as PAMPs, these studies accentuate the question of how hidden or embedded PAMPs are exposed to the corresponding receptors of the innate immune systems. At present, these mechanisms are not fully understood but lytic enzymes of the hosts and, at least in animals, phagocytosis appear to play important roles in releasing PAMPs from their 'hidden' locations.

PAMPs that are characteristic of fungi and oomycetes

Oomycetes and fungi are major classes of plant pathogens. Structures that are hallmarks of fungi include ergosterol, fungal-specific glycosylated proteins, and the wall components chitin and β-glucan. Although the activity of these structures has not been studied in molecular detail in animals, they have all been found to act as PAMPs in plants [6•]. Similarly, cell-wall components that are characteristic of phytopathogenic oomycetes have long been known as potent inducers of plant defence [6•]. The best-studied examples are heptaglucoside, the classic general-elicitor that induces a defence response in soybean, and the conserved Pep13-domain of the cell-wall transglutaminase, which activates resistance responses in *Solanaceae* [27]. Apparently, some of these fungal- and oomycetes-derived patterns are recognized by only a few plant species whereas others, notably chitin, are recognized by all of the higher plant species tested [6•,28].

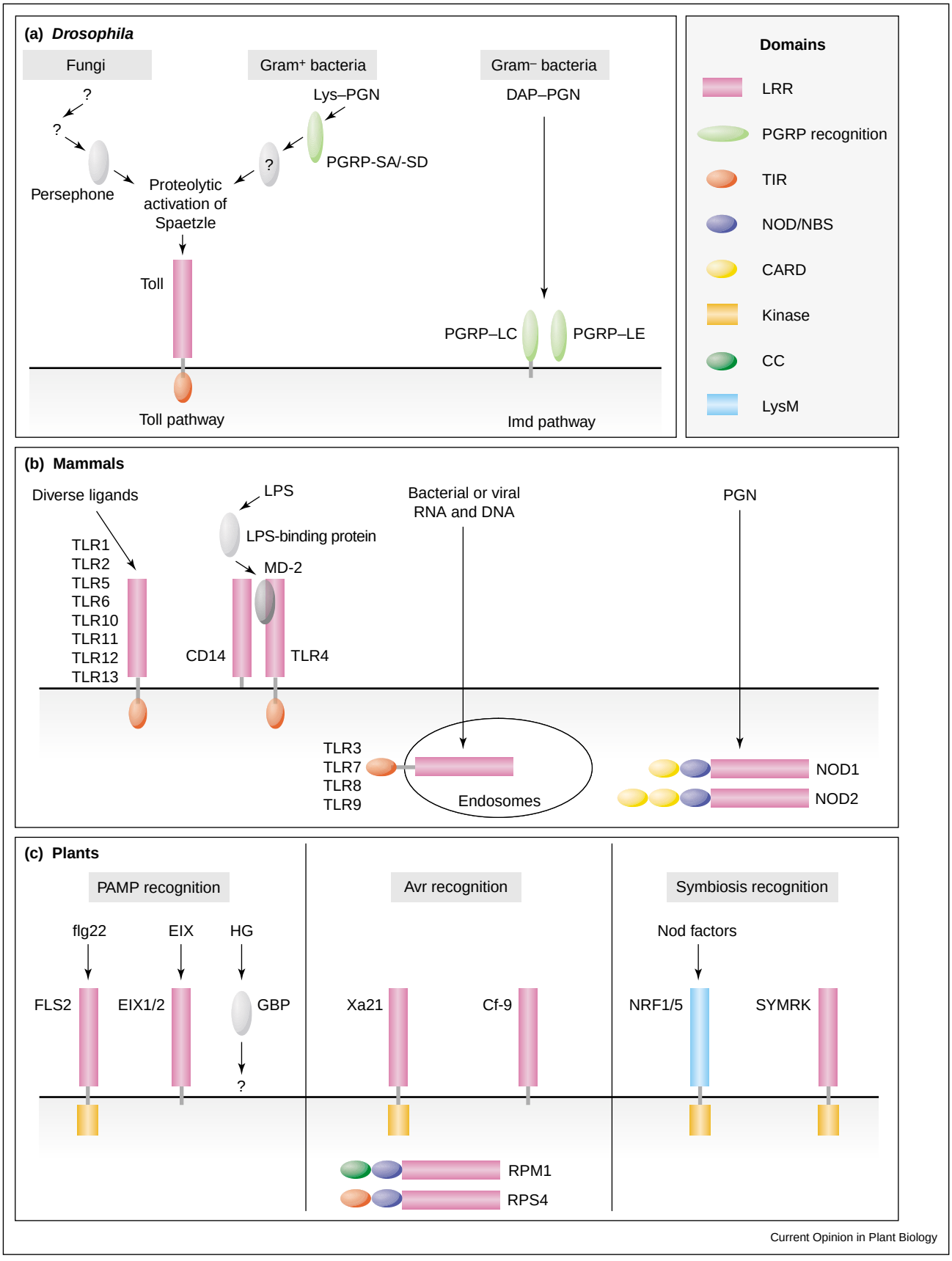
Pattern recognition receptors in the spotlight

Mammalian innate immunity relies on several groups of structurally different transmembrane pattern recognition receptors (PRRs) for the detection of PAMPs [29•,30]. The most prominent group of PRRs comprises the Toll-like receptors (TLRs), a family of a dozen transmembrane proteins containing leucine-rich repeat (LRR) ectodomains that sense bacteria, fungi, protozoa and viruses ([29•]; Figure 2).

Drosophila Toll, the namesake of the TLR family, is a receptor involved in larvae development that responds to the endogenous cytokine Spaetzle ([31,32•]; Figure 2). In the adult fly, however, Toll is essential for defence responses mediated by soluble PRRs that recognize Gram-positive bacteria and fungi [31,32•]. An indirect mechanism is also involved in LPS recognition in mammals in which the soluble LPS-binding protein interacts first with LPS, allowing subsequent interaction of this complex with CD14 and MD-2, and then with TLR4 [7•]. Cooperative interaction with other TLR or non-TLR pattern recognition proteins explains the observation that some TLRs are involved in sensing several structurally different PAMPs [26].

The first PRR protein to be identified in plants is a soluble, cell-wall located protein that specifically binds the classic heptaglucoside elicitor from oomycetes ([33]; Figure 2). Recent data show that this glucan-binding protein has an intrinsic endo-β-glucanase activity [34••]. Astonishingly, homologs of this glucanase seem

Figure 2


(a) Drosophila
Fungi
Gram+ bacteria
Gram− bacteria
?
?
Persephone
Proteolytic activation of Spaetzle
Lys–PGN
?
PGRP-SA/-SD
DAP–PGN
Toll
Toll pathway
PGRP–LC
PGRP–LE
Imd pathway
Domains
LRR
PGRP recognition
TIR
NOD/NBS
CARD
Kinase
CC
LysM
(b) Mammals
Diverse ligands
LPS
LPS-binding protein
MD-2
Bacterial or viral RNA and DNA
PGN
TLR1
TLR2
TLR5
TLR6
TLR10
TLR11
TLR12
TLR13
CD14
TLR4
TLR3
TLR7
TLR8
TLR9
Endosomes
NOD1
NOD2
(c) Plants
PAMP recognition
Avr recognition
Symbiosis recognition
flg22
EIX
HG
Nod factors
FLS2
EIX1/2
GBP
?
Xa21
Cf-9
NRF1/5
SYMRK
RPM1
RPS4
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to be present in diverse plant species, but high-affinity binding and elicitor response to the heptaglucoside is restricted to a few species of the *Fabaceae*. Thus, the receptor component that is involved in transmembrane signalling remains to be identified.

Some TLRs of mammals directly interact with their PAMP-ligands. A well-studied example is TLR5, which binds flagellin [20••,35••]. In *Arabidopsis* plants, flagellin is perceived through its direct interaction with the transmembrane LRR-receptor kinase FLAGELLIN-SENSING 2 (FLS2) ([36,37]; D Chinchilla, Z Bauer, M Regenass, T Boller, G Felix, unpublished; Figure 2). Mutation of the FLS2 protein of *Arabidopsis* leads to loss of flagellin perception and enhanced susceptibility to bacterial infection [38•]. Similarly, a polymorphism that causes a translational stop in one of the TLR5 alleles in humans correlates with increased susceptibility to Legionellosis [39••]. However, other than sharing the common feature of an extracellular LRR domain, there is no obvious sequence similarity between FLS2 and TLR5.

Plants seem to have no clear homologs of TLRs but they have large gene-families that encode receptor-like kinases (RLKs) [40,41] and receptor-like proteins (RLPs) [42]. RLKs are transmembrane proteins that have versatile amino-terminal ectodomains, which are thought to act as recognition sites for extracellular signals, a transmembrane domain and an intracellular kinase domain. RLPs can be defined as RLKs that lack the intracellular kinase domain. Relative to the total number of genes in these families, more than 600 in *Arabidopsis* and more than 1000 in rice, only a few RLKs and RLPs have been assigned specific roles in development, growth, symbiosis and defence [40,41]. Most of the RLPs characterized to date have roles in defence, as exemplified by the *Cf* genes of tomato (Figure 2) and *RPP27* of *Arabidopsis* [40]. Recent work on the tomato receptor for the fungal elicitor xylanase has provided a first example of an RLP that functions as a PRR [43••]. In *Arabidopsis*, the FLS2 protein represents the only RLK currently known to be involved in PAMP perception, but other members of this large family are likely to play similar functions.

Co-evolution of hosts and pathogens: a never-ending play of hide and seek

PAMPs are essential microbial structures that are intrinsically difficult to modify without loss of functionality. Plants and animals recognize multiple PAMPs that signal the same class of microbes. This redundancy probably ensures and potentiates the efficiency of recognition by the hosts and, on the side of pathogens, sets multiple hurdles for strategies to avoid PAMP-based recognition. Nevertheless, as observed for flagellin, a strategy of recognition-avoidance seems to be important for bacterial pathogens of animals [13•]. Similarly, peculiar variations in the sequence of flagellins from some plant-associated bacteria might reflect selection pressure for a non-detectable flg22-domain [22,44].

Apart from hiding or masking their PAMPs, microbial pathogens have evolved other strategies to overcome the ancient forms of PAMP-based defence systems. Suppression of defence by the pathogens is one of these strategies. Microbial secretion systems that directly inject effectors into their host cells are currently a hot topic in the field of plant–pathogen interactions [45,46••,47••]. In turn, some plant species or cultivars have evolved R proteins to detect these effectors, or rather the modifications triggered by them, as summarized by the guard hypothesis [4,5•]. Conceptually, R proteins are related to PRRs. Recent data suggest that cytoplasmic proteins that have a nucleotide oligomerisation domain (NOD) act as receptors for PGN within mammalian cells [23]. Cytoplasmic R proteins of plants, some of which also carrying NOD domains (Figure 2), have long been known to be involved in the detection of Avr products [4,5•]. One can wonder whether some members of the large and rapidly

(Figure 2 Legend) Schematic representation of proteins that are involved in microbe sensing in *Drosophila*, mammals and plants. **(a)** In adult *Drosophila* flies, infection by fungi or Gram-positive bacteria leads to activation of the Toll pathway (as reviewed in [31,32•]). PAMPs from these microorganisms do not directly interact with Toll; rather, PGN from Gram-positive bacteria (Lys-PGN) interacts with PGN-recognition proteins (PGRPs). These complexes activate an as-yet-unknown circulating protease that cleaves Spaetzle. The cleavage product finally triggers Toll signalling. Similarly, Spaetzle can be cleaved by the serine protease Persephone after activation by a process that is triggered in the presence of unknown fungal PAMPs. PGN from Gram-negative bacteria (diaminopilemic acid [DAP]-PGN) binds to different PGN-recognition proteins that trigger the immune deficiency (Imd) pathway. **(b)** Toll-like receptors are the main sensors of PAMPs in mammals. TLR1 recognizes bacterial triacyl lipopeptides. TLR2, in cooperation with TLR1 and TLR6, senses diverse ligands such as lipoteichoic acid and fungal zymosan. TLR5 directly interacts with flagellin, whereas TLR4 requires the formation of a complex with CD14 and MD-2 to percieve LPS that is bound to LPS-binding protein. TLR3, TLR7, TLR8 and TLR9 are present in endosomes and are involved in the perception of single- and double-stranded viral RNA and non-methylated bacterial-DNA. The PAMPs that are recognized by TLR10, TLR11, TLR12 and TLR13 are still unknown. Bacterial peptidoglycans are served by the cytoplasmic proteins NOD1 and NOD2 (reviewed in [26]). **(c)** In plants, only a few receptors or binding proteins for PAMP perception have been identified to date (left panel): FLS2 of *Arabidopsis* binds flagellin [36], EIX1 and EIX2 bind fungal ethylene-inducing xylanase (EIX) and the soybean β-glucan-binding protein (GBP) interacts with the *Phytophthora*-derived heptaglucoside (HG) [33,34••]. Many R proteins that are involved in recognizing different Avr-products are known, and the figure (middle panel) illustrates only a few representative examples: the rice RLK Xa21, which recognizes an unknown Avr product from *Xanthomonas oryzae* pv. *oryzae*; the tomato RLP Cf-9, which is required for perception of the fungal Avr9 from *Cladosporium fulvum*; and RPM1 and RPS4 of *Arabidopsis*, which mediate recognition of AvrRpm1and AvrRps4 from *Pseudomonas syringae* (reviewed in [4,5•]). For the establishment of symbiosis with *Rhizobiaceae* (right panel), legumes such as *Lotus* require the LysM-type RLKs NRF1 and NRF5, prime candidates for receptors that perceive Nod-factor signals. Interestingly, symbiosis receptor kinase (SYMRK) is required for association with both bacterial and fungal symbionts, but its ligand is still unknown (reviewed in [49•]).

evolving families of *R* genes [48] might encode receptors that sense more-general microbial patterns.

Conclusions

The structural and functional similarities of proteins that are involved in innate immune recognition in animals and plants have been interpreted as evidence for evolutionary conservation. However, this conservation refers to modules that are required for the perception of extracellular signals in general. The RLKs of plants use common elements of signal perception and transmembrane signalling to perceive endogenous signals that regulate growth, development and reproduction as well as non-self signals that are important for symbiosis and defence. Thus, the flagellin receptor FLS2 is more closely related to CLAVATA1, which regulates meristem maintenance, than to any of the known animal PRRs.

Recent data point towards the evolution of a convergent repertoire of PAMPs that are detected by different organisms. For example, conserved abundant surface structures of the microbes represent well-suited targets for detection of non-self. The PAMPs that are perceived by different plant species highlight overlapping but non-congruent repertoires, indicating a rather rapid evolution of the corresponding PRRs. It is therefore not surprising that the PAMPs that are recognized by organisms belonging to different kingdoms also differ. In animals, studies on innate immunity have been focused on only a few model organisms, notably humans, mice and *Drosophila*. One can anticipate that a bigger, more diverse, repertoire will emerge from studies with animals from different phyla.

Plants possess a big array of potential receptors, most of them orphan with respect to their functions or ligands. The combination of forward and reverse genetics with biochemistry should allow us to identify new receptors and to understand the molecular basis of receptor activation. Finally, the position of PAMP-based recognition in the disease resistance of plants is not fully established, but future work might further loosen boundaries between Avr and PAMP perception.

Acknowledgements

The work in the authors' laboratory is supported by the Swiss National Science Foundation. The authors apologize to colleagues whose work could not be cited because of space limitations.

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