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.

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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 flagellar inner core and build the filament architecture. The central part
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 [67]. Similarly, cell-wall components that are characteristic of phytopathogenic oomycetes have long been known as potent inducers of plant defence [67]. 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 [67,28].

**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 larval 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
Biotic interactions

Figure 2

(a) Drosophila

Fungi

Gram+ bacteria

Proteolytic activation of Spaetzle

Persephone

Toll pathway

Imd pathway

Domains

LRR

PGRP recognition

TIR

NOD/NBS

CARD

Kinase

CC

LysM

(b) Mammals

Diverse ligands

LPS

Bacterial or viral RNA and DNA

PGN

Endosomes

TLR1 TLR2 TLR5 TLR6 TLR10 TLR11 TLR12 TLR13 CD14 TLR4

(c) Plants

PAMP recognition

flg22 EIX HG

FLS2 EIX1/2 GBP

Avr recognition

Xa21 Cl-9

Nod factors

Symbiosis recognition

NRF1/5 SYMRK

GBP

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 Legionella [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 elictor 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 flag22-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
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.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


The authors map the domain of flagellin recognized by TLR5 to a conserved domain that is buried in the inner part of the flagellar filament. Flagellin and TLR5 co-precipitate, indicating physical interaction between the molecules.


This study identifies the most abundant bacterial protein, EF-Tu, as a PAMP recognized by Arabidopsis and other Brassicaceae. Intriguingly, this perception system recognizes a protein that is genuinely located in the cytoplasm of bacteria.


A historical point of view on TLR discoveries that highlights the importance of TLRs for the immune system of mammals. Evolutionary questions on protein domains that are involved in innate and adaptive immunity are also addressed.


A recent review that summarizes current knowledge on PAMP perception in Drosophila.


A soluble protein was previously identified as receptor-binding site for the heptaglucan elicitor [33]. Here, this glucan-binding protein is shown to have an intrinsic endoglucosidase activity that might be involved in the release of β-glucan fragments during initial contact with Phytophthora.


Together with [20], this study shows direct interaction between flagellin and TLR5.


This paper describes the rapid induction of about 1000 genes and induced disease resistance in Arabidopsis plants treated with flagellin. The biological importance of this PAMP perception is demonstrated by the finding that Arabidopsis mutants that lack the flagellin receptor FLS2 show enhanced susceptibility to bacterial pathogens.


This paper provides evidence that flagellin perception by TLR5 plays a role in disease resistance in humans. Individuals with only one of their TLR5 alleles affected by a stop codon polymorphism apparently suffer from higher susceptibility to Legionnaire’s disease.


EIX is a potent elicitor only in certain cultivars of tomato. By map-based cloning the authors identified the two closely related RLPs LeEIX1 and LeEIX2 as essential for EIX perception. Silencing of the LeEIX genes leads to loss of EIX binding in tomato, whereas heterologous expression leads to gain of EIX binding in tobacco or mammalian cells. Tobacco cells that express LeEIX2 but not LeEIX1 also respond to EIX with a hypersensitive response. Finally, a mutation in the putative endocytosis signal of LeEIX2 abolishes this responsiveness, suggesting that internalization plays a key role in activation of the EIX receptor.


This paper describes the isolation of three Avr genes from *Melampsora lini* that are recognized by the L5, L6 and L7 resistance genes. These Avr genes are expressed in haustoria and encode small secreted proteins. Recognition of these proteins apparently occurs inside plant cells, suggesting that they are delivered into host cells during rust infection by a yet unknown mechanism (see also [47]).


Elegant work characterizing a first Avr gene of the oomycete *Peronospora parasitica*. Both, the Avr gene and the corresponding resistance gene *RPP13*, exhibit extraordinary polymorphism, suggesting a coevolutionary process involving attempts to evade host resistance by the pathogen and the development of new detection capabilities by the plant host. As found for the Avr products of *M. lini* [46], it appears that this Avr product is recognized within the cytoplasm of the plant cells, suggesting a protein transport process from the pathogen to the host.


49. Riely BK, Ane JM, Pennetsa RV, Cook DR: **Genetic and genomic analysis in model legumes bring Nod-factor signaling to center stage.** *Curr Opin Plant Biol* 2004, 7:408-413.

A comprehensive and informative review of the recent findings on the symbiosis of legumes with rhizobial bacteria and mycorrhizal fungi.