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Research Focus

Specific recognition of bacteria by plant LysM domain receptor kinases

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The symbiosis between rhizobial bacteria and leguminous plants is based on the exchange of specific signal molecules between both partners. The molecular basis of recognition of lipochitin oligosaccharide signal molecules that are secreted by these symbiotic bacteria has been revealed in recent reports. It was shown that specific recognition involves several family members of the serine/threonine receptor kinase family that contain LysM motifs in their extracellular domains. These LysM domain receptors appear to be crucial for the earliest responses of the host plant *Lotus japonicus* to infection by rhizobia. In another host plant, *Medicago truncatula*, it was shown that LysM domain receptors are also involved in specific recognition during later steps in bacterial infection.

Various genera of Gram-negative soil bacteria, collectively called rhizobia [1], are able to engage in a symbiotic relationship with plants of the legume family. The result of this host-specific symbiosis is the formation of a specialized plant organ, known as the root nodule, which is invaded by the rhizobia. Bacteria inside the cells of the root nodules differentiate into a specialized form that is able to fix atmospheric nitrogen and are therefore beneficial for the plant. During the past decade the process of signal exchange that underlies the infection and root nodulation process has been studied extensively. As a result, many bacterial signal molecules and their roles in triggering various specific responses from the host plant have been identified [2]. Bacterial lipochitin oligosaccharides (LCOs, also called Nod factors), which are responsible for triggering the formation of infection threads and the root nodule, have been the major focus of research to date. Recently, information on plant receptor proteins that are involved in the recognition of LCOs has been published, demonstrating the function of various members of the family of receptor serine/threonine kinases [3–7].

Receptor kinases involved in microbial recognition

The studies by Endre *et al.* [4] and Stracke *et al.* [3] showed that mutations in genes that encode receptor kinases containing extracellular leucine rich repeats (LRR) result in a host plant that is unable to form root nodules after inoculation with rhizobia. These mutants were also shown to be unable to form a symbiosis with arbuscular mycorrhizal fungi that improve the uptake of inorganic nutrients, most prominently phosphate. However, early responses of the mutant roots, such as root hair deformation (indicative of the first step in the infection process), were still observed. Therefore, it was concluded that these LRR receptor kinases, known as SYMRK (symbiosis receptor-like kinase) or NORK (nodulation receptor kinase), were involved in a common pathway that evolved early in evolution to regulate intracellular symbiotic plant–microbe interactions. The LRR domain of these receptors is related to the extracellular domains of other plant kinases that are involved in defence against pathogens and also the animal Toll-like receptors that function in the innate immune system (Figure 1). This shows that the microbial recognition systems based on LRR receptors evolved more than 1.8 billion years ago from a common eukaryotic ancestor [8,9].

Studies of the symbiotic LRR receptors also indicate that specific recognition of the rhizobial symbionts requires additional factors that can discriminate between different symbiotic partners. Madsen *et al.* [6] and Radutoiu *et al.* [7] showed that other plant mutants, which are completely unable to respond to rhizobial innoculation with root hair deformation, contain mutations in another sub-family of serine/threonine receptor kinases. These receptors are characterized by the presence of two or three LysM motifs in their extracellular domain. The model legume *Lotus japonicus* that was used in these studies contains two such genes that are essential for the infection and root nodulation process: *NFR1* and *NFR5*. Mutants in the *NFR5* gene have a stronger phenotype then mutants in the *NFR1* gene; root hairs of the latter mutants responded to Nod factors only by alkalinization or acidification. By contrast, mutants in the *NFR5* gene do not display any measurable electrophysiological response in their root hairs after stimulation with Nod factors. The difference in the phenotype of these two genes could be correlated with the lack of an activation loop in the intracellular kinase domain of the NFR5 receptor, suggesting that initial NFR5 kinase activity is activated after Nod factor perception, and subsequently activates the NFR1 protein within a heterodimeric complex (Figure 1).

In the study of Limpens *et al.* [5], which was performed in the model legume *Medicago truncatula*, another representative of the LysM domain receptor kinase was identified. RNA interference studies for this gene, called *LYK3*, showed that it also plays a role in the rhizobial–plant symbiotic process. However, in contrast to the mutant phenotypes of the related genes in *L. japonicus*, the phenotype resulting from the inactivation of the *LYK3* gene is not very strong; it displays normal early and late

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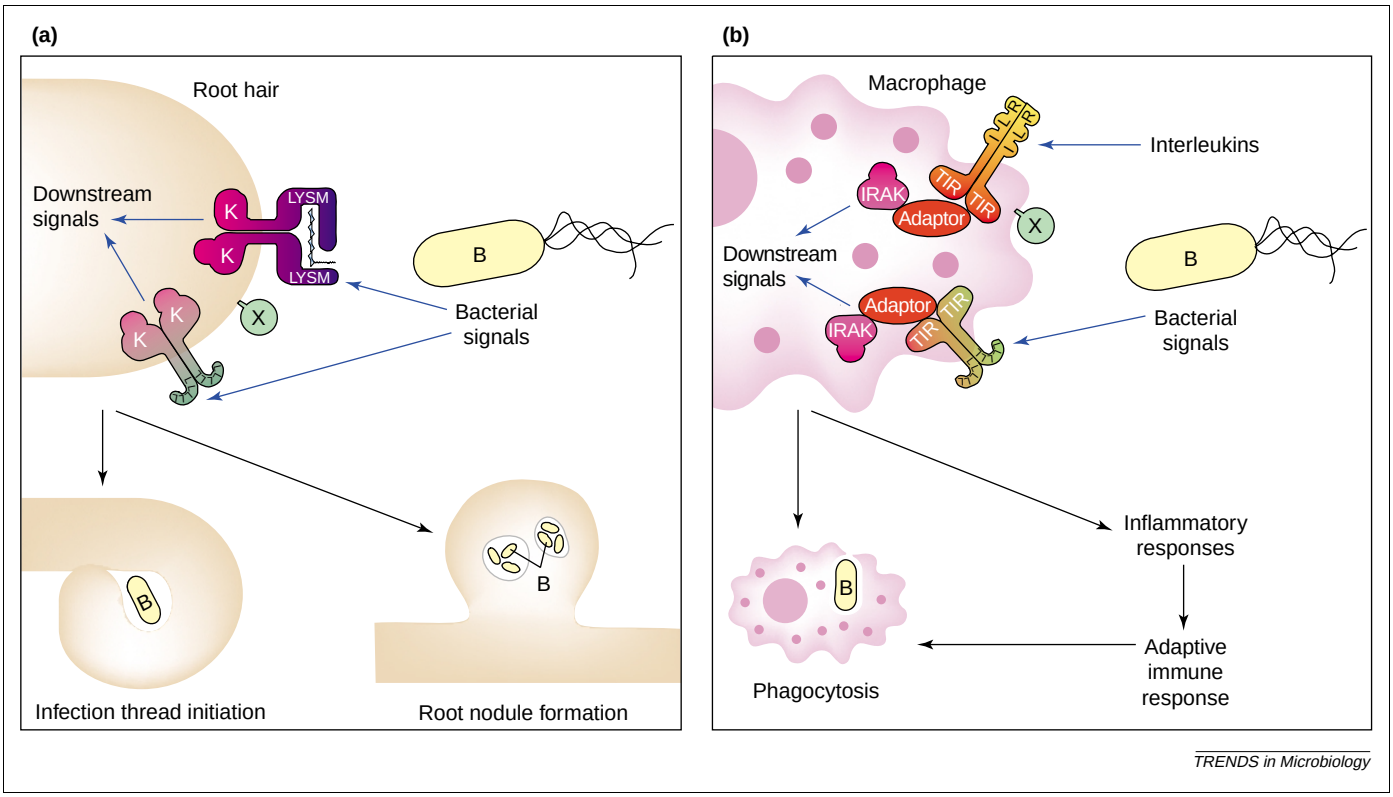

Figure 1. A model for the recognition of bacterial signal molecules by plants and a comparison with the innate immune response in vertebrates. Molecular or cellular components are not drawn on scale. **(a)** A scheme for the response of root hairs of leguminous plants to rhizobial signal molecules. Recognition of Nod factors by the LysM domain-containing receptors NFR1 and NFR5 (purple) and the leucine-rich repeat (LRR) domain-containing receptor SYMRK (green domain) is probably assisted by other unknown recognition factors (indicated with 'X'). **(b)** A scheme for the recognition of bacterial factors by white blood cells, such as macrophages. A direct link between phagocytosis and bacterial recognition via the Toll-like receptors (in addition to the established role of the complement system and adaptive immune system) has recently been reported by Doyle and colleagues [14]. Various extracellular proteins that assist the Toll-like receptors (green) in recognizing bacterial factors, termed X, have been identified in the human system, for instance, the CD14 protein. Abbreviations: B, bacterium; ILR, interleukin receptor; K, kinase domain; TIR, Toll–interleukin (IL)-1 receptor homology domain.

responses to rhizobial infection, such as root nodulation and infection thread formation. A strong effect as a result of mutations in the *LYK3* gene was only observed when roots were inoculated with mutant bacterial strains that produced aberrant Nod factors. Because the symbiotic phenotype of these mutant bacteria on wild-type plants was not affected, it was concluded that the *LYK* gene plays a role in the rhizobial infection process.

A model for plant recognition of rhizobial Nod factors

On the basis of the results described previously, a dual control mechanism was proposed for recognition of rhizobial Nod factors (Figure 1). In this model the SYMRK receptors are involved in a signal transduction pathway that is a common basis for the recognition of rhizobial bacteria and arbuscular mycorrhizal fungi. By contrast, the LysM domain receptors play a role in the specific recognition of rhizobia. Correlation of the phenotypes resulting from mutations in both types of receptors with the stream of events that follow inoculation of host plants with suitable guest bacteria shows that the LysM domain receptors can be placed 'upstream' of the LRR receptor signal transduction cascade. The fact that mutations in the *NFR5* gene result in plants that are totally unresponsive to rhizobial inoculation but can still undergo a normal symbiosis with mycorrhizal fungi also shows that the function of the SYMRK receptor pathway is independent of activation of the NFR5 pathway. However, it is also clear that there must be several links between these pathways because the mycorrhizal and rhizobial symbioses share various mechanistic similarities and common downstream gene activation targets, such as the activation of several nodulin genes. Furthermore, the intracellular kinase domains of the two classes of receptors are structurally related (Figure 2) suggesting that they share similarities in the downstream signal transduction pathways.

The mechanism of activation in both types of receptors is far from clear. In the case of the SYMRK receptor, an indirect recognition mechanism was proposed based on structural analyses of the LRR domains suggesting a function in protein recognition [10]. This mechanism is supported by the observation that the related animal Toll-like receptors often function indirectly in the recognition of microbial signal molecules, such as lipopolysaccharide (LPS). This would explain why the SYMRK receptor is able to function in the recognition of both rhizobia and mycorrhiza, which are not expected to share common signal molecules. In the case of the LysM domain receptors, there are indications that they could be involved in the direct recognition of rhizobial Nod factors, because studies on autolysin from *Lactococcus lactis* suggest that the LysM domain is directly involved in binding of peptidoglycan [11]. Furthermore, LysM domains are often associated with chitinase domains in various proteins of algae and nematodes. Although this is not the case in the

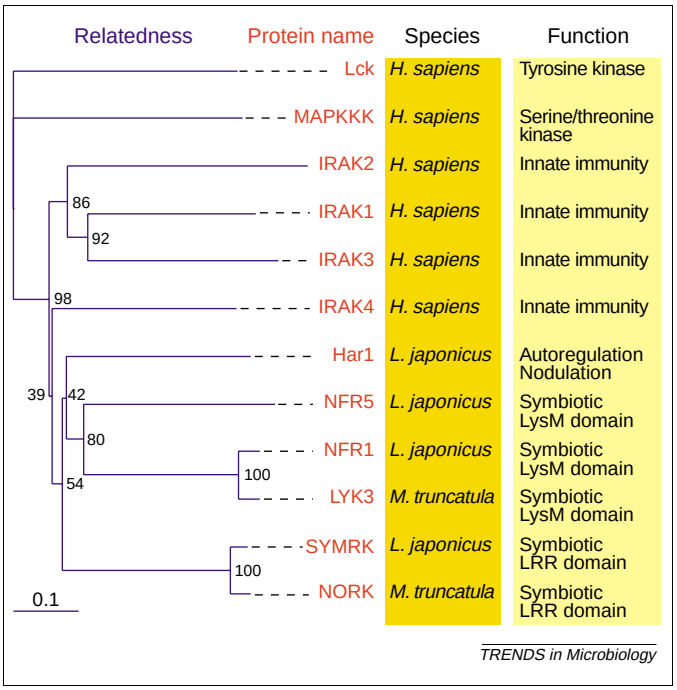

Figure 2. Phylogenetic tree and summary of functions of the receptor kinase domains. The chosen examples are plant receptors that have been shown to be involved in microbe recognition and non-receptor kinases that are involved in signal transduction in the human innate immune system. The receptor Har1 [15] and its homologue in soybean [16] are involved in shoot control of root development and nodulation. The human tyrosine kinase Lck and a mitogen-activated protein kinase (MAPKKK) have been chosen as the outgroup. Shown is a tree rooted to the LcK kinase and constructed by neighbour-joining analysis based on the alignment of the amino acid sequences of the kinase domains. The numbers indicate the occurrence of nodes during bootstrap analysis shown as percentages of 10 000 reiterations. The scale bar indicates the branch length. GenBank accession numbers: Lck, NP_005347; MAPKKK, NP_004712; IRAK1, NP_001560; IRAK2, NP_001561; IRAK3, NP_009130; IRAK4, AAH13316; Har1, CAD42335; NFR5, CAE02597; NFR1, CAE02589; LYK3, AAQ73155; SYMRK, AAM67418; and NORK, AAM76685 or CAD10809.

identified NFR5, NFR1 and LYK3 receptors, it indicates that the presence of LysM domains is generally associated with binding of glycans.

Evolutionary lessons from plant–microbe recognition factors

The discovery that receptors involved in symbiosis contain a serine/threonine kinase domain is not surprising, considering that these domains are greatly abundant in the plant kingdom; for example, there are up to 1000 members in the model plant *Arabidopsis* [12]. The LRR receptor kinases constitute a large proportion of this group, with up to 180 members in *Arabidopsis*. The LRR domain of these receptors is similar to the extracellular domain of the animal Toll-like receptors, indicating an evolutionary conserved function of the LRR domain in the recognition of microbes [8,9]. In addition, downstream signaling components of the plant and animal innate immune systems that are regulated by LRR receptors show strong similarities. The serine/threonine kinase domain of the plant receptors is highly conserved in the interleukin (IL)-1 receptor-associated kinases (IRAKs; Figure 2), which have important functions in the animal innate immune system by linking the Toll-like and interleukin receptors to downstream components. Furthermore, the intracellular domain of these receptors is also found in plant intracellular proteins that are indicated to play a role in the innate immune system of plants [8]. LysM domain receptors are much less abundant, although they are of widespread occurrence throughout the plant kingdom. In humans, there are no counterparts that share a combination of a LysM domain with a serine/threonine kinase domain. However, recent results for the plant receptors indicate that various human genes of unknown function that encode LysM domains [e.g. found in the genome database (http://www.ncbi.nlm.nih.gov)] might also function in microbial recognition in the innate immune system. It is possible that these proteins interact with kinase proteins, such as those belonging to the IRAK family, or with Toll-like receptors (Figure 1).

Concluding remarks

The discovery of plant receptors that are involved in the recognition of rhizobial bacteria presents a major breakthrough in understanding root nodule symbiosis. It also has major implications for understanding the interactions of plants with arbuscular mycorrhizal fungi. Further unraveling of the downstream components of the signaling pathways that are governed by these receptors can be expected to follow soon as full use can be made of various two-hybrid techniques. Furthermore, the similarities between the identified receptors and counterparts in other model plants that are genetically more accessible than legumes, such as *Arabidopsis* and rice, will make advanced comparative studies possible. The impact of future studies on these pathways will have even broader implications outside plant–microbe interactions, because relatedness of domains of these receptors with counterparts in animal models and humans (e.g. IRAK kinases; Figure 1) will warrant functional comparative studies that can also shed further light on the evolution of the signal transduction pathways underlying the innate immune system.

The most difficult challenge that the rhizobial–plant interaction field is now facing is the identification of structural features of plant receptors that determine the specificity for bacterial trigger factors, which have been shown to be very diverse [2]. In this respect, little support can be expected from comparative studies, considering the limited progress in the identification of structural motifs that are involved in determining the protein-binding specificity for carbohydrates [13]. However, if direct binding studies of Nod factors advance further, use can be made of heterologous expression studies using identified receptor proteins in other model systems that are biochemically or genetically more accessible than plants.

References

1 Spaink, H.P. *et al.* (1998) The *Rhizobiaceae*: Molecular biology of model plant-associated bacteria, Kluwer Academic Publishers, Dordrecht
2 Spaink, H.P. (2000) Root nodulation and infection factors produced by rhizobial bacteria. *Annu. Rev. Microbiol.* 54, 257–288
3 Stracke, S. *et al.* (2002) A plant receptor-like kinase required for both bacterial and fungal symbiosis. *Nature* 417, 959–962
4 Endre, G. *et al.* (2002) Cloning of a receptor kinase gene regulating symbiotic nodule development. *Nature* 417, 962–966
5 Limpens, E. *et al.* (2003) LysM domain receptor kinases regulating rhizobial Nod factor-induced infection. *Science* 302, 630–633

6 Madsen, E.B. *et al.* (2003) A receptor kinase gene of the LysM type is involved in legume perception of rhizobial signals. *Nature* 425, 637–640
7 Radutoiu, S. *et al.* (2003) Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. *Nature* 425, 585–592
8 Dangl, J.L. and Jones, J.D. (2001) Plant pathogens and integrated defence responses to infection. *Nature* 411, 826–833
9 Spaink, H.P. (2002) Plant-microbe interactions: a receptor in symbiotic dialogue. *Nature* 417, 910–911
10 Kobe, B. and Kajava, A.V. (2001) The leucine-rich repeat as a protein recognition motif. *Curr. Opin. Struct. Biol.* 11, 725–732
11 Steen, A. *et al.* (2003) Cell wall attachment of a widely distributed peptidoglycan binding domain is hindered by cell wall constituents. *J. Biol. Chem.* 278, 23874–23881
12 The Arabidopsis Genome Initiative, (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408, 796–815
13 van der Holst, P.P.G. *et al.* (2001) Proteins involved in the production and perception of oligosaccharides in relation to plant and animal development. *Curr. Opin. Struct. Biol.* 11, 608–616
14 Doyle, S.E. *et al.* (2004) Toll-like Receptors Induce a Phagocytic Gene Program through p38. *J. Exp. Med.* 199, 81–90
15 Krusell, L. *et al.* (2002) Shoot control of root development and nodulation is mediated by a receptor-like kinase. *Nature* 420, 422–426
16 Searle, I.R. *et al.* (2003) Long-distance signaling in nodulation directed by a CLAVATA1-like receptor kinase. *Science* 299, 109–112

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The multiple routes of MHC-I cross-presentation

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Cross-presentation of phagocytosed antigens on major histocompatibility (MHC) class I molecules is known to occur via two alternative routes; one involving processing and loading of the antigenic peptides inside a vacuolar compartment, the other requiring processing inside the cytosol and loading to MHC-I in the endoplasmic reticulum. A new pathway has been described recently in which antigens are transported to the cytosol, processed by phagosome-associated proteasomes and returned back to the phagosome for MHC-I loading.

Most cells express at their surface a large number of major histocompatibility class I (MHC-I) molecules, each hauling on a groove a short peptide resulting from the physiological degradation of the cellular protein content. When viruses or bacteria infect cells, some of the MHC-I peptide-binding sites become occupied by peptides that are derived from microbial proteins. These microbial peptide–MHC-I complexes are recognized by pre-activated $CD8^+$ cytotoxic T lymphocytes (CTL), which curtail the spread of the infection by killing the diseased cells [1]. Because CTL can potentially inflict severe autoimmunity, their activation must be tightly controlled. This is partly achieved by limiting the origin of the activating signal to a subset of bone marrow-derived cells, collectively known as professional antigen-presenting cells (pAPCs) [2–4]. Similar to all other cells, infected pAPCs can present endogenously synthesized microbial peptides. In this pathway, the peptides are mostly generated from ubiquitinated proteins by proteasomal digestion in the cytosol. They are then transferred to the endoplasmic reticulum (ER) by the a peptide transporter known as TAP (transporter associated with antigen presentation) and loaded into the groove of newly synthesized MHC-I with the intervention of other ER proteins, such as tapasin, calreticulin and Erap57 (which constitute the MHC-I peptide-loading machinery). The peptide–MHC-I complexes are finally conveyed to the cell surface via the secretory pathway [5,6].

MHC-I cross-presentation as an alternative to pAPC infection

In contrast to all other cells, pAPCs can also present antigens garnered from the extracellular milieu [7]. This process, known as cross-presentation, has been shown to induce CTL responses to viruses that do not infect pAPCs [3], or that upon infection block direct presentation [8,9]. Furthermore, recent data indicate that cross-presentation contributes substantially to overall CTL responses against vesicular stomatitis virus (VSV) and Sendai virus [10]. Although pAPCs are capable of cross-presenting soluble antigens [11–13], cross-presentation is enhanced by up to 10 000-fold when the same antigen is bound to micron-sized beads and acquired by phagocytosis [14]. Similarly, bacteria [15] and virus-infected cells [16,17] can be phagocytosed and the microbial antigens cross-presented on MHC-I. Two routes have previously been described for the cross-presentation of phagocytosed antigens. In the cytosolic route, the antigen is somehow transferred from phagosomes into the cytosol to follow steps similar to those of the direct pathway because TAP is required for transport into the ER and is blocked by Brefeldin A, an inhibitor of the secretory pathway [17–19] (Figure 1, number 3). In the vacuolar route, antigen-processing is performed by proteolytic enzymes at some stage in the phagocytic pathway and the peptides are loaded into MHC-I molecules that have been recycled from the cell membrane [15] (Figure 1, number 4). This pathway does not require TAP transport and is not inhibited by brefeldin A. Recent publications in Nature by Houde *et al.* [20] and Guermonprez *et al.* [21] and a paper in PNAS by Ackerman *et al.* [22] report a third route whereby antigens can be cross-presented.

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