REVIEW

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Plant signalling in symbiosis and immunity

Cyril Zipfel[1] & Giles E. D. Oldroyd[2]

Plants encounter a myriad of microorganisms, particularly at the root–soil interface, that can invade with detrimental or beneficial outcomes. Prevalent beneficial associations between plants and microorganisms include those that promote plant growth by facilitating the acquisition of limiting nutrients such as nitrogen and phosphorus. But while promoting such symbiotic relationships, plants must restrict the formation of pathogenic associations. Achieving this balance requires the perception of potential invading microorganisms through the signals that they produce, followed by the activation of either symbiotic responses that promote microbial colonization or immune responses that limit it.

The establishment of a homeostatic phytobiome (the interaction of a plant with its immediate environment and its microbial communities) requires the plant to be able to sense its surroundings and to respond appropriately[1]. Plants can recognize microbial molecules[2], which ultimately leads to mutualism (referred to here as symbiosis, for simplicity) or immunity. The restriction of invading organisms as governed by immune signalling is effective against all types of plant pathogens and pests, including viruses, insects, nematodes and even parasitic plants[3–7]. By contrast, the establishment of symbiotic associations is restricted to a few species of bacteria and fungi. These processes are governed by the perception of both pathogen-associated molecular patterns (PAMPs) and symbiotic signals. The most widespread (present in most land plants) and evolutionarily ancient symbiosis in plants is the relationship with arbuscular mycorrhizal fungi of the phylum Glomeromycota, which helps to provide the plant with nutrients such as phosphorus[8]. Several plants, including members of the family Fabaceae (legumes), have developed a root symbiosis with nitrogen-fixing bacteria, of the genus *Frankia* or rhizobia, that provide access to the main source of nitrogen in the atmosphere, which is too inert for use in most biological processes[8]. Both of these symbioses are intracellular: arbuscular mycorrhizal fungi are accommodated in specialized host-membrane compartments in root cortical cells, forming arbuscules, and rhizobia are accommodated in root-derived organs called nodules. Other symbiotic associations exist in the plant kingdom but, in most cases, the signalling mechanisms that govern these symbioses are unknown. In this Review, we will therefore focus on the association of plants with arbuscular mycorrhizal fungi and rhizobia.

In the past 10 years, exciting discoveries have been made about the identity of the receptors and ligands involved in the perception of microorganisms by plants, the molecular mechanisms of these perception events and the downstream signalling components[9,10]. Here, we summarize our knowledge of the early perception and signalling events of immunity and symbiosis, with special emphasis on receptor kinase complexes and calcium signalling. We also discuss the strategies that symbionts use to avoid or overcome the plant immune response and examine biotechnological efforts that aim to engineer broad-spectrum disease resistance and root-nodule symbiosis in crops, both of which are required for a more sustainable and resilient agriculture.

Immune and symbiotic receptors are similar yet distinct

Plants use cell-surface receptors to perceive their immediate external environment. In the past decade, the receptors involved in the perception of pathogenic or symbiotic microorganisms have been revealed, highlighting similarities but also differences between the recognition events that they facilitate.

The immunogenic perception of bacteria

Plant recognition of microbial signals, whether they be PAMPs or symbiotic signals, involves complexes of cell-surface receptor kinases. PAMP recognition receptors can be either receptor kinases, which possess an extracellular domain that is involved in ligand perception, a single-pass transmembrane domain and an intracellular kinase domain, or receptor-like proteins that have an extracellular domain but lack an intracellular signalling domain[10] (Fig. 1). Archetypal bacterial molecules that are recognized by the plant immune system include flagellin, elongation factor Tu (EF-Tu), lipopolysaccharides and peptidoglycans[3]. Although the recognition of microbial molecules across the plant kingdom is partially conserved, at least some of this conservation is the result of convergent evolution to recognize widely conserved bacterial proteins through the recognition of various epitopes by distinct receptors. For instance, flagellin and EF-Tu both display several immunogenic epitopes, including the conserved 22-amino-acid epitope flg22 that is recognized by most land plants through the leucine-rich repeat (LRR) receptor kinase FLS2 (ref. 3). However, the tomato plant *Solanum lycopersicum* (as well as other species that belong to the family Solanaceae) and rice (*Oryza sativa*) can perceive further flagellin epitopes in a FLS2-independent manner[11]. Similarly, plants from the family Brassicaceae can perceive the EF-Tu-derived 18-amino-acid epitope elf18 through the LRR-receptor kinase EFR[3], whereas rice plants recognize another epitope[12]. The wider conservation of these PAMPs would render nitrogen-fixing rhizobial bacteria susceptible to immune detection during initial contact with legumes, which could impede the establishment of symbiosis[13]. However, rhizobial-derived flg22 is not immunogenic because important residues that are required for its recognition are not conserved[3], which suggests that rhizobia have evolved to evade the recognition of this potent PAMP.

The perception of *N*-acetylglucosamine derivatives

The recognition of fungal and oomycete pathogenic species by plants is dominated by the perception of the main components of the cell wall:

[1]The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK. [2]Department of Cell and Developmental Biology, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK.

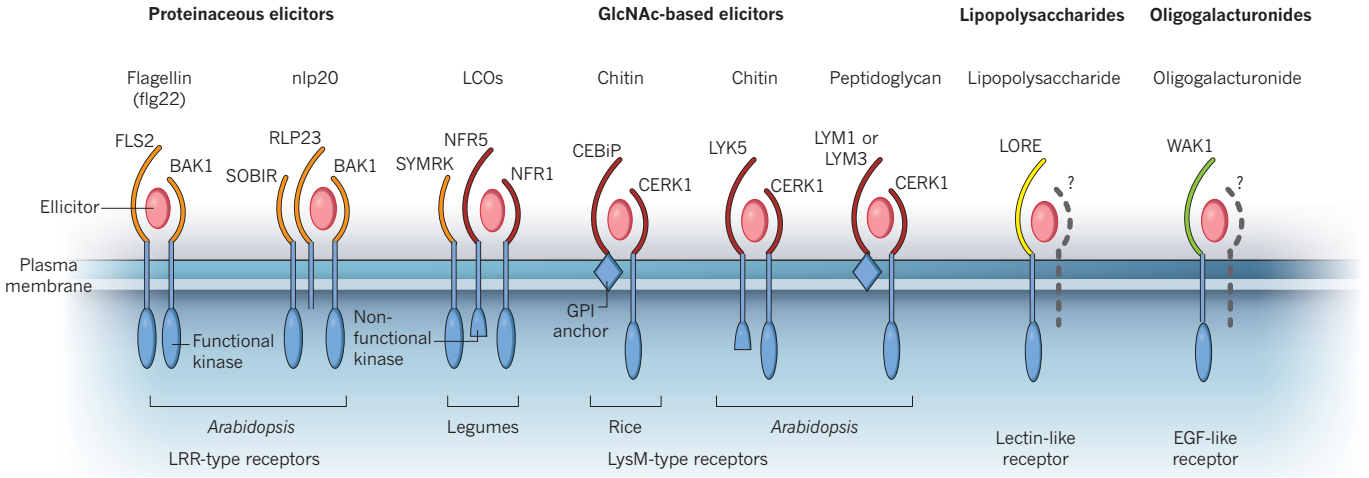

Figure 1 | LysM-receptor-kinase complexes that are involved in the sensing of microorganisms. Classes of the main receptor complexes that are involved in the perception of microbial signals are shown. In general, proteinaceous elicitors (for example, flg22 from flagellin and nlp20, an epitope that is conserved in bacteria, fungi and oomycetes) are perceived by receptors with LRR-type extracellular domains (orange) that also associate with LRR-type co-receptors. GlcNAc-based elicitors such as LCOs, chitin and peptidoglycan are bound by LysM motifs (red) that are present in receptor complexes. Further classes of receptors that are associated with the recognition of lipopolysaccharides (lectin-like receptors, yellow) and oligogalacturonides (EGF-type receptors, green) are also being discovered. Each receptor complex contains at least one receptor-like kinase with either functional or non-functional kinase motifs. The complexes can also include receptor-like proteins that contain a transmembrane domain and an extracellular domain but that lack an intracellular signalling domain (for instance, RLP23 in the nlp20 receptor), or extracellular proteins that are anchored to the membrane by glycosylphosphatidylinositol (GPI) (for instance, CEBiP in the chitin receptor of rice and LYM1 and LYM3 in the peptidoglycan receptor of *Arabidopsis*). EGF, epidermal growth factor.

chitin in fungi and β-glucans in oomycetes. Because chitin recognition is widespread in plants, including mosses[3,14], the perception of chitin must have been an early evolutionary innovation. Chitin oligosaccharides are β-1,4-linked polymers of *N*-acetylglucosamine (GlcNAc) with various degrees of polymerization (Fig. 2a). Chitin oligosaccharide hexamers or octamers are potent inducers of immune responses[3]. Bacterial peptidoglycans, which are chief constituents of the cell walls of Gram-positive and Gram-negative bacteria, are structurally similar to chitin oligosaccharides but carry alternating β-1,4-linked GlcNAc and *N*-acetylmuramic acid residues, with peptide linkers between the two polymeric heteroglycan chains (Fig. 2b). Strikingly, the microbial signals that are necessary for the establishment of arbuscular mycorrhizal symbioses (the Myc factors) and root-nodule symbioses (the Nod factors) are also derivatives of chitin oligosaccharides. An important difference, however, is the presence of lipid modifications on the chitin-oligosaccharide backbone of these symbiotic signals, which transforms them into lipochitooligosaccharides (LCOs) (Fig. 2c). Rhizobia produce a diversity of modified Nod-LCOs that differ in the length of their chitin-oligosaccharide chain, lipid acylation and the presence of modifications such as sulfation, acetylation and fucosylation, which probably contribute to plant host specificity[15,16,17]. Notably, a role for chitin oligosaccharide tetramers (chitotetraose) and heptamers in arbuscular mycorrhizal symbiosis has also been reported: these molecules induce calcium oscillations in the cell nucleus, which is a hallmark of early arbuscular mycorrhizal and root-nodule symbiotic signalling[8], in various legumes (pea (*Pisum sativum*), *Medicago truncatula* and *Lotus japonicus*) and rice[18–21]. The initiation of arbuscular mycorrhizal symbiosis therefore probably involves the coordinated perception of a mixture of LCO and chitin oligosaccharide molecules. The similarities between chitin and peptidoglycan and symbiotic chitin oligosaccharides and LCOs also raise intriguing questions about how plants distinguish chitin oligosaccharide-containing molecules that trigger immunity from those that trigger symbiosis.

The roles of LysM receptors

The perception of GlcNAc-containing microbial molecules by plants involves receptor kinases or receptor-like proteins that carry lysin motif (LysM)-domain-containing extracellular domains[22]. In *Arabidopsis thaliana*, chitin is perceived by a ligand-induced heteromeric complex that is composed of the LysM-receptor kinases CERK1 and LYK5 (as well as the LYK5 paralogue LYK4), in which LYK5 serves as a high-affinity binding receptor[23] (Fig. 1). In rice, the mechanism of chitin perception differs slightly because CERK1 does not form a ligand-induced complex with a LysM-receptor kinase. Instead, it associates with the glycosyl phosphatidylinositol-anchored LysM-receptor-like protein CEBiP, which is the main high-affinity chitin-binding site[23] (Fig. 1). Interestingly, in *Arabidopsis*, one of three CEBiP orthologues (LYM2) also plays a part in chitin perception, in which it controls the chitin-induced closure of plasmodesmata (cytoplasmic conduits that traverse plant cell walls to enable intercellular continuity) and resistance to fungal pathogens in a CERK1-independent manner[24–26], indicating that further chitin-receptor complexes must exist. Although the other *Arabidopsis* CEBiP orthologues (LYM1 and LYM3) do not have a role in chitin perception[24], they bind peptidoglycan and probably form a heteromeric complex with CERK1 to mediate peptidoglycan-induced immune responses[27] (Fig. 1). Surprisingly, the rice LysM-receptor-like proteins LYP4 and LYP6 function as receptors for both chitin and peptidoglycan by forming a ligand-induced complex with CERK1 (refs 28–30).

Consistent with their biochemical similarity to chitin, Nod-LCOs are perceived by pairs of LysM-receptor kinases in legumes, as occurs in the perception of chitin in *Arabidopsis* (Fig. 1). In *Lotus*, this complex is composed of NFR1 and NFR5, but in *Medicago* it contains the orthologous proteins LYK3 and NFP[9]. Notably, NFR5 and NFP are non-functional kinases, which suggests that they require NFR1 or LYK3 to be present in their respective heteromeric receptor complexes to function during root-nodule symbiosis[9].

Since the discovery of Myc-LCOs[17], researchers have been hunting for their corresponding receptors. Given the molecular similarities between Nod-LCOs and Myc-LCOs, as well as the fact that early arbuscular-mycorrhizal and root-nodule symbiotic signalling share components (that form the common symbiotic pathway), it was proposed that the receptors involved in perceiving both types of LCO are similar[8,17]. Indeed, NFP seems to be involved in Myc-LCO-induced lateral-root formation, transcriptional changes and nuclear calcium oscillations[17,20,31]; however, it is not required for mycorrhization[32–34] or Myc-chitin-oligosaccahride-induced nuclear calcium oscillations[18,20]. The suggestion that a LysM-receptor kinase that is closely related to NFP or NFR5 is involved in Myc-LCO perception is supported by

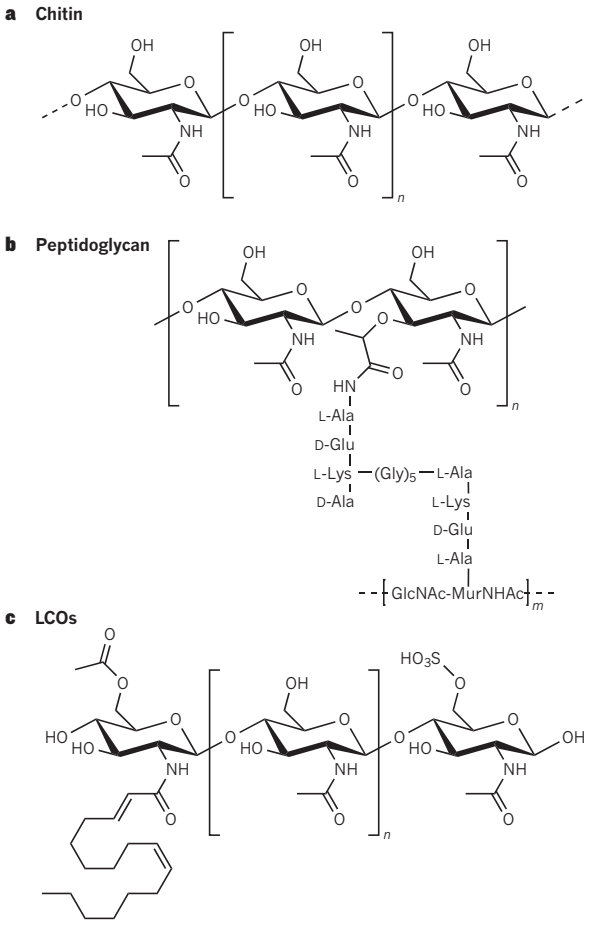

Figure 2 | Similarities between the *N*-acetylglucosamine-containing microbial molecules that are recognized by plants. **a**, Chitin is a simple molecule that consists of repeating units of *N*-acetylglucosamine (GlcNAc). **b**, Peptidoglycan possesses a backbone that is made of alternating GlcNAc and muramic acid residues, with peptide linkers that connect to further sugar chains. **c**, LCOs have a chitin backbone but also possess an acyl group that is attached to the non-reducing terminal GlcNAc. The LCOs that are produced by various rhizobia and arbuscular mycorrhizal fungi possess further modifications, such as the sulfation on the reducing terminal GlcNAc that is shown here.

the finding that the knockdown of an NFP orthologue in *Parasponia andersonii* (the only non-leguminous plant that can achieve root-nodule symbiosis with rhizobia) affects both arbuscular-mycorrhizal and root-nodule symbioses[35]. Furthermore, the orthologue of NFP and NFR5 in tomato (SILYK10), but not their orthologue in rice (RLK2), seems to be required for successful arbuscular mycorrhizal colonization[36,37]. NFP, NFR5 and RLK2 have several paralogues in their respective species (*Medicago*, *Lotus* and rice), which implies that the lack of a clear phenotype in their mutants may be the result of genetic redundancy. Among these paralogues, in *Medicago* LYR1 and LYR3 are potential candidates for the Myc-LCO receptors. Indeed, *LYR1* expression is upregulated in mycorrhization[38–40] and LYR3 has a high affinity for LCOs and interacts dynamically with the Nod-LCO receptor LYK3 in response to LCOs[41–43], which suggests potential functions in the mycorrhizal association. NFR1 and LYK3 may also be involved in facilitating the arbuscular mycorrhizal symbiosis as their respective mutants are partially impaired during mycorrhization[34]. Interestingly, NFR1 and LYK3 are orthologous to rice CERK1, which was shown to be involved in the arbuscular mycorrhizal symbiosis, as well as having a well-documented role in immunity[34,37]. By contrast, CEBiP, the main chitin-binding receptor in rice, is not required for the arbuscular mycorrhizal symbiosis[37]. This suggests that several receptor complexes may be involved in Myc-LCO perception. Furthermore, possibly owing to the heterogeneity of Myc-LCOs that are produced by a given arbuscular mycorrhizal fungus and also to the functional redundancy of LysM-receptor kinases that are involved in Myc-LCO perception, no definitive candidates for the Myc-LCO receptors have yet emerged. Genetic redundancy may be complicated further by the fact that arbuscular mycorrhizal fungi produce both LCOs and chitin oligosaccharides. The receptor for chitotetraose produced by arbuscular mycorrhizal fungi is still unknown, and it is possible that successful arbuscular mycorrhizal colonization requires the coordinated perception of Myc-LCOs and Myc-chitin oligosaccharides.

CERK1 is a common co-receptor for LysM receptors

The many roles of CERK1 in the perception of chitin, peptidoglycans and Myc-LCOs indicate that this protein is used as a common co-receptor by various high-affinity ligand-binding LysM-containing receptor kinases and receptor-like proteins, and that it has a dual function in both immunity and symbiosis. Interestingly, the Tyr-Ala-Gln or Tyr-Ala-Arg motifs in NFR1, LYK3 and most CERK1 orthologues in non-leguminous plants that form arbuscular mycorrhizal symbioses has been proposed to correlate with the ability to function in such a symbiosis but is not required for immunity[44–46]. It will be interesting to test whether NFR1 and LYK3 play a part in the perception of chitin or peptidoglycans, or whether they show a more global involvement in immunity, as suggested by the observation in *Lotus* that Nod-LCOs can induce the expression of immune-related genes in an NFR1-dependent manner[46]. Also, the transient overexpression of NFR1 and NFR5, LYK3 and NFP, or CERK1 in *Nicotiana benthamiana* induces cell death — a response that is often associated with the induction of immune responses — in a manner that is dependent on functional NFR1 and LYK3 kinase domains[47–49]. Notably, NFP also seems to be involved in immunity because its expression can be correlated positively with resistance to several fungal and oomycete pathogens[50,51]. Conversely, it has been proposed that some filamentous pathogens hijack components of symbiosis to facilitate host-root colonization[52,53]. It is therefore becoming clear that symbiotic and immune signalling pathways are more intertwined than was thought previously, which further highlights the need to understand the molecular basis for the specificity in signalling that ultimately leads to the plant's decision to let friends (symbionts) enter but leave foes (pathogens) at the door.

Other symbiotic signals

As well as symbiotic chitin oligosaccharides and LCO molecules, other oligosaccharides, including lipopolysaccharides, exopolysaccharides, cyclic β-glucans and peptidoglycans are known to be important for the establishment of the root-nodule symbiosis[54]. Although some of these molecules are proposed to help rhizobia to cope with immune responses or environmental stresses, exopolysaccharides of varying compositions and structures can contribute to host specificity[54,55]. Importantly, a LysM-receptor kinase, EPR3, was identified in *Lotus* that mediates the direct recognition of exopolysaccharides from compatible rhizobial strains[56]. Interestingly, expression of the gene *EPR3* is induced upon perception of Nod-LCO, which suggests a two-step mechanism: first, rhizobia are sensed by the roots of legumes through NFR1–NFR5- or LYK3–NFP-mediated Nod-LCO perception to initiate root-nodule symbiotic signalling, which is followed by the EPR3-dependent sensing of exopolysaccharides that further controls compatibility and colonization[56]. This finding highlights the many important roles played by LysM-receptor kinases in root symbioses.

Activation of symbiotic and immune receptor complexes

The downstream transmission of signals from receptor complexes involves a variety of processes, with notable similarities between the immunity and symbiosis signalling pathways. The recognition of PAMPs by plant cells leads to a series of cellular events, including the production of apoplastic reactive oxygen species, an increase in the concentration of cytosolic calcium, the activation of cytosolic mitogen-activated protein (MAP) kinase and calcium-dependent protein kinase (CDPKs) cascades, and transcriptional changes[3]. Most studies on

signalling in symbiosis have focused on oscillations in nuclear calcium concentrations, which regulate the expression of symbiotic genes[8,9]. However, Nod-LCO recognition also activates a transient increase in reactive oxygen species and an influx of calcium across the plasma membrane, both of which are associated with the tips of growing root hairs[57,58]. These lesser-studied components of symbiosis signalling show parallels with PAMP signalling (Fig. 3).

The formation of ligand–induced receptor kinase complexes

At the molecular level, components acting downstream of activated PAMP receptors that induce immune responses are starting to be uncovered in detail in both *Arabidopsis* and rice (see ref. 10 for a comprehensive review on this topic). A common theme is that ligand-bound PAMP receptors form stable complexes with co-receptors that have ectodomains containing similar biochemical domains (such as the LRR or LysM) in a ligand-dependent manner. For example, in *Arabidopsis*, the LRR-receptor kinase FLS2 forms a flg22-dependent complex with the LRR-receptor kinase BAK1, and the LysM-receptor kinase LYK5 forms a chitin-dependent complex with CERK1; however, in rice the LysM-receptor-like protein CEBiP forms a complex with CERK1 upon chitin binding[10]. LRR-receptor-like proteins such as RLP23 also form complexes with BAK1 (or related somatic embryogenesis receptor-like kinases, SERKs) upon binding of ligands such as nlp20 but require a further LRR-receptor kinase called SOBIR1 to do so[10] (Fig. 1).

As mentioned previously, CERK1 in rice and its orthologues in legumes have a clear role as co-receptors that form part of LCO-perceiving and chitin-oligosaccharide-perceiving LysM-receptor complexes in symbioses. Although these findings highlight a potential similarity between immune and symbiotic signalling, they are more likely to reflect common molecular strategies that are used by similar biochemical classes of ligand-binding receptors for their ligand-induced activation; for example, LRR-type receptors use LRR-type co-receptors such as BAK1, whereas LysM-type receptors engage LysM-type co-receptors such as CERK1). This is demonstrated further by the fact that BAK1 and SERK proteins function as co-receptors for LRR-receptor kinases not only involved in immunity, but also in growth and development[59].

The role of regulatory receptor kinases

Immune and symbiotic receptor complexes also contain regulatory receptor kinases. For instance, the LRR-receptor kinase BIR2 negatively regulates the formation of a complex between FLS2 and BAK1 in *Arabidopsis*[60], whereas the *Arabidopsis* malectin-like LRR-receptor kinase IOS1 is a positive regulator of this formation[61]. IOS1 also regulates responses to chitin through an unknown mechanism[61] and the

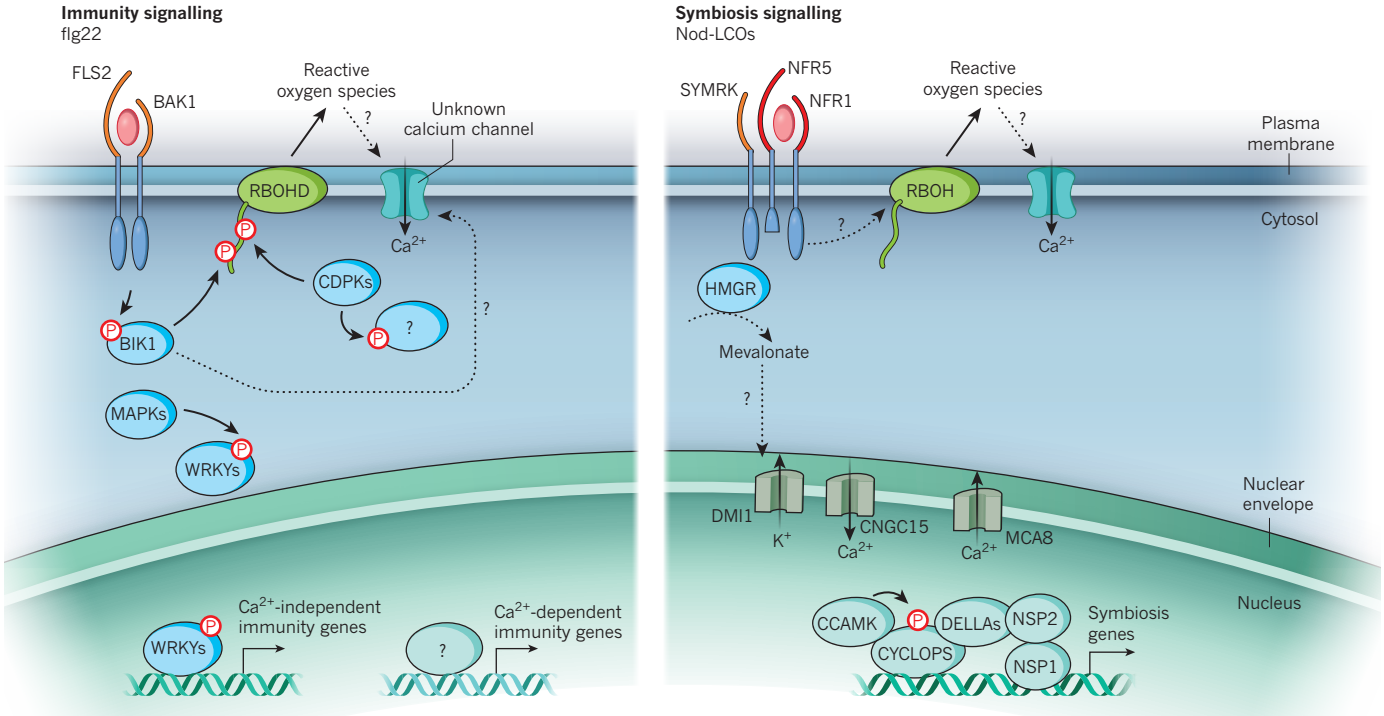

Figure 3 | Plant immune and symbiotic intracellular signalling pathways. A simplified model of immunity signalling (left) and symbiosis signalling (right) in plants, which shows the predominant components of these signalling pathways. For immunity signalling, the pathway for the perception of flagellin epitope flg22 is shown. Recognition of flg22 by the FLS2–BAK1 receptor complex leads to the phosphorylation of the receptor-like cytoplasmic kinase BIK1, which then phosphorylates the N terminus of the NADPH oxidase RBOHD, leading to the production of reactive oxygen species. This release is proposed to activate an unknown calcium channel that is located on the plasma membrane, causing an influx of calcium at the cytosol that is recognized by CDPKs, which further phosphorylate RBOHD and enhances its activation. CDPKs may also phosphorylate other targets that are thought to be involved in the activation of genes involved in plant immunity. In parallel to the calcium-mediated pathway, a MAP kinase (MAPK) pathway leads to the phosphorylation of WRKY transcription factors that control aspects of immunity gene expression. Symbiosis signalling involves the recognition of Nod-LCOs by a receptor complex of the LysM receptor kinases NFR1 and NFR5 and the LRR-containing receptor kinase SYMRK (known as DMI2 in *Medicago*). Pathway activation leads to a burst of reactive oxygen species and an influx of calcium across the plasma membrane, similar to that observed in immune signalling. The NADPH oxidase RBOH and the plasma-membrane-localized calcium channel that are involved in symbiosis signalling have not yet been defined. The recognition of LCOs (and chitin oligosaccharides) also activates calcium oscillations in the nucleus. The 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) that associates with SYMRK is involved in the production of mevalonate, which may function as a secondary messenger to the nucleus. Several channels that are located at the nuclear membrane coordinate the release of calcium from the nuclear envelope and endoplasmic reticulum: a complex of DMI1 (known as POLLUX in *Lotus*) and CNGC15 regulate counterflows of potassium and calcium, enabling these ions to flow without impinging on membrane polarity, and the calcium ATPase MCA8 pumps calcium back into the nuclear envelope. Nuclear calcium oscillations activate CCAMK, which phosphorylates CYCLOPS to promote the induction of symbiosis gene expression. CYCLOPS may form part of a large complex that contains a number of GRAS-domain-containing transcription factors (NSP1, NSP2 and DELLA) that are also necessary for the expression of symbiosis genes.

Arabidopsis CERK1-interacting LRR-receptor kinase LIK1 negatively regulates chitin and flg22 responses[62].

Similarly, symbiotic receptors interact with the orthologous malectin-like LRR-receptor kinases SYMRK (in *Lotus*) and DMI2 (in *Medicago*) that are required for both arbuscular mycorrhizal and rhizobial interactions[63,64] (Fig. 3). SYMRK associates with NFR5 and NFR1 and seems to activate downstream signalling[65,66]. The overexpression of *SYMRK* is sufficient to induce nodule formation and arbuscular mycorrhizal-related gene expression[66]. An interaction between SYMRK and the MAP kinase kinase SIP2 has also been reported[67]. Furthermore, an interaction between DMI2 and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) 1, a key enzyme of the mevalonate pathway, seems to be associated with the activation of nuclear calcium oscillations, because the application of mevalonate alone is sufficient to activate symbiotic nuclear calcium spiking and gene expression[68]. Interestingly, the mevalonate pathway has been shown to be important during the innate immune response in mammals[69] and this may reflect functions that are common to both symbiosis and immunity signalling.

Cytoplasmic receptor–like kinases as signal transducers

An emerging underlying principle of plant receptor-kinase-based signalling is the dynamic association and activation of receptor-like cytoplasmic kinases (RLCKs), which are phylogenetically related to receptor kinases but lack the transmembrane and extracellular domains, as the direct, downstream substrates of receptor-kinase complexes. For example, several members of the RLCK subfamily VII, including BIK1, PBL1, PBL27 and PCRK1 in *Arabidopsis* or RLCK176 and RLCK185 in rice, associate dynamically with PAMP receptors and are positive regulators of immune signalling[30,70–74]. Interestingly, although some RLCKs (for example, BIK1) can act downstream of both LRR-type and LysM-type PAMP receptors, the regulatory function of others (such as PBL27) is more restricted[10]. Distinct RLCKs connect ligand-activated receptor complexes to appropriate downstream signalling outputs. For example, BIK1 interacts with and phosphorylates the *Arabidopsis* plasma membrane NADPH oxidase RBOHD following elicitation by flg22, elf18 or chitin[75,76]. However, BIK1 is not required for flg22-induced MAP kinase activation[77]. Conversely, *Arabidopsis* PBL27 and rice RLCK185 are required for chitin-induced MAP kinase activation but not for the production of reactive oxygen species[72,73,78]. An RLCK has been demonstrated to be involved in nodulation but its exact role in symbiotic signalling has not been ascertained[79].

Calcium signalling in symbiosis and immunity

Calcium is a common component of immunity and symbiosis signalling pathways. However, the cellular location and nature of these calcium signals differ, which probably reflects mechanistically distinct calcium encoding and decoding machinery.

Generation of symbiotic nuclear calcium signals

Calcium oscillations in the nucleus are a hallmark of symbiotic responses[8] and a number of studies have deciphered the components that are required for this signalling output. As mentioned previously, mevalonate is sufficient to induce symbiotic calcium spiking in the nucleus, and this involves the cation channels DMI1 (in *Medicago*) or CASTOR and POLLUX (in *Lotus*), which are located at the nuclear envelope[68]. Although these channels are necessary for calcium spiking, the identity of the calcium channel itself was unknown until 2016. In *Medicago*, the cyclic nucleotide-gated channels CNGC15a, CNGC15b and CNGC15c, which are found on the nuclear membrane, have been shown to interact with DMI1 and to directly mediate nuclear calcium oscillations in response to Nod-LCOs and Myc-LCOs[80]. The release of calcium from the nuclear envelope is probably a function of the CNGC15 proteins, with DMI1 repolarizing the membrane through the transport of potassium into the nuclear envelope[81] and nuclear calcium homeostasis being maintained through the action of the calcium ATPase MCA8 (ref. 82).

Decoding symbiotic nuclear calcium signals

Symbiotic oscillations in the calcium concentration of the nucleus are decoded by the nuclear-localized calcium and calmodulin-dependent protein kinase CCAMK in both *Lotus* and *Medicago*[82–84]. CCAMK can bind calcium directly through EF-hand domains or indirectly through a calmodulin-binding domain. Binding of calcium to the EF-hand domains suppresses the action of CCAMK by activating autophosphorylation, which promotes a hydrogen-bond network that stabilizes the inactive state of the protein[85,86]. Mutations that interfere with this hydrogen-bond network create autoactive forms of CCAMK that can spontaneously activate rhizobial and mycorrhizal responses in the plant[86–88]. Calmodulin binding overides the CCAMK inactive state by blocking autophosphorylation and promoting substrate phosphorylation[85]. Because calcium only binds to calmodulin during symbiotic calcium oscillations, the calmodulin-mediated activation of CCAMK will occur only in the symbiotically activated cell. The main substrates for CCAMK are the transcriptional activators CYCLOPS in *Lotus* and IPD3 in *Medicago*[89,90]. CCAMK-induced phosphorylation of CYCLOPS is sufficient to activate nodulation[91], which is analogous to gain-of-function mutations in CCAMK[87,88]. CYCLOPS is probably a component of a much larger transcriptional complex that contains at least three GRAS-domain-type transcription factors (NSP1, NSP2 and DELLA) for nodulation signalling[92,93] or the DELLA proteins and unknown transcription factors for mycorrhizal signalling[94]. The DELLA proteins play many parts in plant development and their involvement in symbiosis provides a means to coordinate symbiosis with development through the plant hormone gibberellin[95,96]. Interestingly, DELLA proteins also function during immunity in plants[97], which provides a possible mechanism of cross-talk between immunity and symbiotic signalling pathways.

Cytoplasmic symbiotic calcium signals

Most research on symbiosis signalling has focused on oscillations in the calcium concentration of the nucleus. But in legumes, Nod-LCOs can also induce changes in the calcium concentration of the cytosol[57,58], through the movement of calcium across the plasma membrane. These changes originate at the tip of growing root hairs and correlate both spatially and temporally with Nod-LCO-induced changes in reactive oxygen species[58,98]. The role of this transient release of reactive oxygen species as a positive activator of symbiosis is supported by evidence to show that silencing of NADPH oxidases in *Medicago* blocks infection by rhizobia[99,100]. Interestingly, activation of the cytosolic calcium influx requires higher concentrations of Nod-LCOs than does the activation of nuclear calcium oscillations[101]. This implies a function for the cytosolic calcium influx during rhizobial infection, after the bacteria have become trapped in a curled root hair and are therefore increasing in number in an enclosed compartment. Although the molecular mechanisms of this Nod-LCO-induced transient release of calcium are poorly understood, the correlations between reactive oxygen species and calcium suggest the involvement of mechanisms that are comparable to those of PAMP signalling (Fig. 3).

Calcium signals in immunity signalling

Although nuclear calcium spiking is often presented as a defining output of symbiotic signalling, PAMP perception can also induce oscillatory changes in nuclear calcium concentrations[102,103]. Also, PAMP-induced changes in cytosolic calcium concentrations were also shown to be of an oscillatory nature using single-cell analysis[104,105]. Whereas the molecular mechanisms that underlie PAMP-induced changes in calcium concentrations are still mostly unknown, both cyclic nucleotide-gated channels and ionotropic glutamate-like receptors have been proposed as candidates for PAMP-induced calcium channels[106,107]. In *Arabidopsis*, decoding of the PAMP-induced calcium response involves at least four CDPKs that are necessary for transcriptional reprogramming during immunity signalling[108]. These CDPKs contribute to the PAMP-activated production of reactive oxygen species[108], for example, through the CPK5-mediated phosphorylation of RBOHD[109]. RBOHD

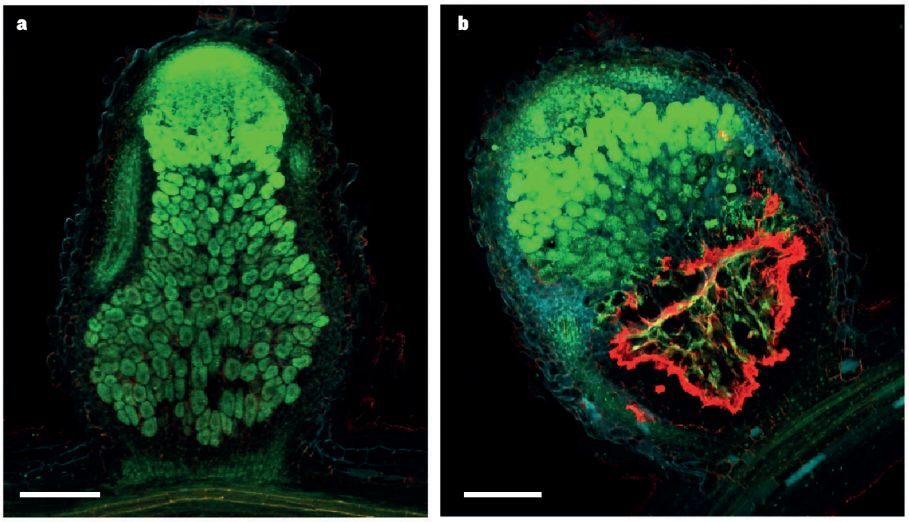

Figure 4 | Nodulation involves the dampening of plant defences. **a**, **b**, Root nodules in wild-type *Medicago truncatula* (**a**) and the *Medicago* 7Y mutant (**b**), which is unable to restrict plant defences in cells that have been infected by rhizobia (**a**, **b**, scale bar, 250 mm). Nucleic acids in both plant cells and bacteria are stained with the dye SYTO 13 (green). Phenolic compounds (autofluorescence, red), which indicate activated plant defences, accumulate in the tissues in **b**. Images courtesy of P. Kalo.

can therefore be phosphorylated by both BIK1 and CPK5 during immunity signalling[75,76]. It has been proposed that the PAMP-induced changes in cytosolic calcium are biphasic, with an initial phase that is independent of reactive oxygen species followed by a reactive-oxygen species-dependent second phase[110]. A simple model has been suggested in which BIK1 activation by the PAMP receptor complex triggers the phosphorylation of RBOHD, which activates the enzyme and releases an initial burst of reactive oxygen species. This promotes the activity of a plasma-membrane-associated calcium channel that releases calcium into the cytosol, which activates CDPKs to promote further RBOHD activity through phosphorylation (Fig. 3). This circuit involving reactive oxygen species and calcium has the potential to amplify the local perception of an invading microorganism, and such amplification is important for guarding other regions of the plant from further microbial invasion[109]. Salt stress can induce long-distance signalling through waves of calcium flux, and such signalling is potentiated by an interplay between calcium and reactive oxygen species[111,112]. Local PAMP perception probably also promotes long-distance signalling throughout the plant using the same interplay.

Regulation of immunity by beneficial microorganisms

The colonization of host plants by microorganisms requires the regulation of immunity signalling. In pathogenic associations, this regulation is mainly the function of microbial effector molecules. Such effectors are similarly involved in the regulation of immunity signalling during the establishment of symbiotic associations, but plants also seem to directly suppress immunity signalling following the recognition of symbiotic microorganisms.

Recognition that is dependent on the expression of plant resistance genes can limit the range of rhizobial species that can establish symbiosis with the host[113], which implies that the activation of plant defences restricts rhizobial colonization. To avoid such defences during symbiosis, symbionts can evade recognition by the plant (as discussed previously) or actively suppress the defences through the action of effectors. Both rhizobia and arbuscular mycorrhizal fungi produce effectors that promote colonization of their host plants[114,115]. Because mutations in the type III secretion system (a mechanism that delivers bacterial effectors into the plant cell) of rhizobia alter the range of hosts that are available to these bacteria, in both positive and negative ways[114], it is clear that such effectors promote rhizobial colonization. However, recognition of these effectors by the host plant can promote immunity, which restricts rhizobial colonization. The conversion of a species of pathogenic bacteria into one that is symbiotic requires the experimental transfer of the ability to make Nod-LCOs in conjunction with the mutation of effectors that restrict bacterial recognition[116]. Interestingly, the role of effector proteins produced by some species of rhizobia are not limited to the regulation of defences. A species from the genus *Bradyrhizobium* that can colonize soybean (*Glycine max*) has the ability to do so even in the absence of Nod-LCO production or recognition, and this function is conferred by an effector that is delivered through a type III secretion system[117]. This effector probably activates symbiotic signalling, which therefore negates the need for Nod-LCO perception. A similar mechanism may explain other examples in which nodulation occurs in the absence of Nod-LCO production[118].

As well as the suppression or evasion of defences by the symbiont, the plant also plays an important part in regulating its own defences during symbiosis. Such regulation seems to take two forms: a direct suppression of immunity signalling that occurs early in the interaction with rhizobia (and presumably arbuscular mycorrhizal fungi) and a later suppression of defences in the nodule. Expression profiling that reveals a large but transient suppression of defence-related genes in the first 24 hours of treatment with rhizobia or Nod-LCO clearly indicates that plant defences are downregulated during symbiosis[119]. This is probably the result of a direct suppression of immunity signalling that follows the recognition of Nod-LCO and chitotetraose and may take the form of a reduction in the level of PAMP receptors on the cell surface[120]. Interestingly, this suppression of immunity signalling by Nod-LCOs and chitotetraose is present in *Arabidopsis*, even though the species does not form root-nodule or arbuscular-mycorrhizal symbioses, and may reflect the importance of plant defences during the production of stable microbiomes and endophytic associations[121]. The end result of the nitrogen-fixing symbiosis is a nodule with cells that have been colonized intracellularly by rhizobial bacteria. Such colonization seems to require a further level of regulation of plant defences — a suggestion that is supported by evidence from a suite of genes in which certain mutations cause the inappropriate activation of plant defences in the nodule[122–125] (Fig. 4). A genetic pathway is emerging for this later regulation of plant defences, which involves a receptor kinase[122], a phospholipase C[126] and a protein of unknown function that resides in the endoplasmic reticulum[125]. It remains to be shown exactly how these genetic components function together to regulate plant defences.

Improving immunity and symbiosis in crops

Now that a considerable level of understanding of the mechanisms that underpin signalling in immunity and symbiosis exists, we are in a position to modify these processes to engineer crops for better performance in the field. PAMP receptors confer broad-spectrum resistance to disease and several have been cloned from economically important crops[7,127–131]. Interestingly, a large number of PAMP receptors seem to be restricted to certain taxonomic groups[2,3,127], which most probably reflects the evolution of receptor kinase and receptor-like protein families through duplication, expansion and divergence[132,133]. As well as

being useful for the identification of PAMP receptors, this knowledge is facilitating efforts to improve disease resistance in crops through the transfer of PAMP receptors (by means of classical breeding or genetic engineering) between species, genera, families or classes of plants to add new recognition specificity to the arsenal of PAMP receptors that are already present in the recipients. The successful transgenic transfer of several PAMP receptors has now been described in several species[7,134–142] and this represents a promising biotechnological approach to engineering broad-spectrum disease resistance in crops. Notably, the efficacy and durability of the disease resistance that is conferred by PAMP-receptor engineering are yet to be demonstrated in field trials. Furthermore, it will be interesting to test the impact of introducing new PAMP receptors on symbioses and on the microbiome. The detailed definition of phytobiomes may enable the use of isolated beneficial strains, individually or as synthetic communities, to improve the resistance of plants to biotic or abiotic stresses[1].

A more challenging venture is to transfer nitrogen-fixing symbiosis from legumes to cereal crops[143]. Similar to the transfer of disease resistance between species, engineering nitrogen-fixing symbiosis in cereals will almost certainly require the transfer of recognition specificities, which will probably involve the transfer of symbiotic receptors. However, nodulation involves more than just the recognition of rhizobial bacteria, and the developmental pathways that are associated with rhizobial infection and nodule organogenesis will probably also need to be transferred to enable effective levels of nitrogen fixation in cereal crops. An alternative approach to engineering nitrogen-fixing cereal crops is the transfer of the bacterial enzyme nitrogenase, which is responsible for nitrogen fixation[143]. Nitrogenase is a complex oxygen-sensitive enzyme with unique cofactors that must also be engineered into the plant. However, the first stages of transfering the components of nitrogenase have delivered promising results[144,145].

It is foreseeable that a combination of the expression of ectopic PAMP receptors, modifications to microbial communities and the expansion of nitrogen-fixing capabilities to other species of crops will contribute to the development of a more sustainable and resilient agriculture in the future.

Concluding remarks

Recent insights have provided a detailed understanding of the mechanisms that underlie signalling pathways in immunity and symbiosis in plants. Although gaps in our knowledge remain, such as the nature of the plasma-membrane-associated calcium channels that are responsible for calcium influx following the perception of PAMP and symbiosis signals, these are likely to be filled in the next few years. However, there are also more challenging questions that relate to the interplay between immunity and symbiosis signalling, which include how these signalling processes impart specific outcomes and what happens when a cell is challenged by many diverse signals. In its natural habitat, a plant is exposed to a wide variety of microorganisms at the root and shoot and must decide how and when to respond. Immunity and symbiosis signalling must contribute to the nature of the microbiome, but the degree to which these signalling processes can structure plant-associated microbial communities is only beginning to be explored. However, there is no doubt that such studies will provide exciting insights into the regulation by plants of their biotic environment. ■

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1. Müller, D. B., Vogel, C., Bai, Y. & Vorholt, J. A. The plant microbiota: systems-level insights and perspectives. *Annu. Rev. Genet.* **50,** 211–234 (2016).
2. Cook, D. E., Mesarich, C. H. & Thomma, B. P. Understanding plant immunity as a surveillance system to detect invasion. *Annu. Rev. Phytopathol.* **53,** 541–563 (2015).
3. Boller, T. & Felix, G. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu. Rev. Plant Biol.* **60,** 379–406 (2009).
4. Niehl, A., Wyrsch, I., Boller, T. & Heinlein, M. Double-stranded RNAs induce a pattern-triggered immune signaling pathway in plants. *New Phytol.* **211,** 1008–1019 (2016).
5. Manosalva, P. *et al.* Conserved nematode signalling molecules elicit plant defenses and pathogen resistance. *Nature Commun.* **6,** 7795 (2015).
6. Prince, D. C., Drurey, C., Zipfel, C. & Hogenhout, S. A. The leucine-rich repeat receptor-like kinase BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED KINASE 1 and the cytochrome P450 PHYTOALEXIN DEFICIENT 3 contribute to innate immunity to aphids in *Arabidopsis*. *Plant Physiol.* **164,** 2207–2219 (2014).
7. Hegenauer, V. *et al.* Detection of the plant parasite *Cuscuta reflexa* by a tomato cell surface receptor. *Science* **353,** 478–481 (2016).
This paper shows that the perception of parasitic plants utilizes PAMP recognition in a way that is similar to the perception of microbes.
8. Oldroyd, G. E. Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nature Rev. Microbiol.* **11,** 252–263 (2013).
9. Limpens, E., van Zeijl, A. & Geurts, R. Lipochitooligosaccharides modulate plant host immunity to enable endosymbioses. *Annu. Rev. Phytopathol.* **53,** 311–334 (2015).
A comprehensive review on the perception of symbiotic chitin oligosaccharides and LCOs by legumes.
10. Couto, D. & Zipfel, C. Regulation of pattern recognition receptor signalling in plants. *Nature Rev. Immunol.* **16,** 537–552 (2016).
A comprehensive review on the mechanisms of plant immune perception and signalling.
11. Fliegmann, J. & Felix, G. Immunity: flagellin seen from all sides. *Nature Plants* **2,** 16136 (2016).
12. Furukawa, T., Inagaki, H., Takai, R., Hirai, H. & Che, F. S. Two distinct EF-Tu epitopes induce immune responses in rice and *Arabidopsis*. *Mol. Plant Microbe Interact.* **27,** 113–124 (2014).
13. Lopez-Gomez, M., Sandal, N., Stougaard, J. & Boller, T. Interplay of flg22-induced defence responses and nodulation in *Lotus japonicus*. *J. Exp. Bot.* **63,** 393–401 (2012).
14. Bressendorff, S. *et al.* An innate immunity pathway in the moss *Physcomitrella patens*. *Plant Cell* **28,** 1328–1342 (2016).
This study reveals that the perception of chitin is evolutionarily conserved in mosses.
15. Dénarié, J., Debellé, F. & Promé, J.-C. *Rhizobium* lipo-chitooligosaccharide Nodulation factors: signaling molecules mediating recognition and morphogenesis. *Annu. Rev. Biochem.* **65,** 503–535 (1996).
16. Lerouge, P. *et al.* Symbiotic host-specificity of *Rhizobium meliloti* is determined by a sulphated and acylated glucosamine oligosaccharide signal. *Nature* **344,** 781–784 (1990).
17. Maillet, F. *et al.* Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. *Nature* **469,** 58–63 (2011).
18. Genre, A. *et al.* Short-chain chitin oligomers from arbuscular mycorrhizal fungi trigger nuclear Ca^{2+} spiking in *Medicago truncatula* roots and their production is enhanced by strigolactone. *New Phytol.* **198,** 190–202 (2013).
19. Oldroyd, G., Mitra, R. M., Wais, R. & Long, S. Evidence for structurally specific negative feedback in the Nod factor signal transduction pathway. *Plant J.* **28,** 191–199 (2001).
20. Sun, J. *et al.* Activation of symbiosis signaling by arbuscular mycorrhizal fungi in legumes and rice. *Plant Cell* **27,** 823–838 (2015).
21. Walker, S. A., Viprey, V. & Downie, J. A. Dissection of nodulation signaling using pea mutants defective for calcium spiking induced by Nod factors and chitin oligomers. *Proc. Natl Acad. Sci. USA* **97,** 13413–13418 (2000).
22. Gust, A. A., Willmann, R., Desaki, Y., Grabherr, H. M. & Nurnberger, T. Plant LysM proteins: modules mediating symbiosis and immunity. *Trends Plant Sci.* **17,** 495–502 (2012).
23. Shinya, T., Nakagawa, T., Kaku, H. & Shibuya, N. Chitin-mediated plant-fungal interactions: catching, hiding and handshaking. *Curr. Opin. Plant Biol.* **26,** 64–71 (2015).
24. Shinya, T. *et al.* Functional characterization of CEBiP and CERK1 homologs in *Arabidopsis* and rice reveals the presence of different chitin receptor systems in plants. *Plant Cell Physiol.* **53**, 1696–1706 (2012).
25. Faulkner, C. *et al.* LYM2-dependent chitin perception limits molecular flux via plasmodesmata. *Proc. Natl Acad. Sci. USA* **110**, 9166–9170 (2013).
26. Narusaka, Y. *et al.* Presence of LYM2 dependent but CERK1 independent disease resistance in *Arabidopsis*. *Plant Signal. Behav.* **8,** (2013).
27. Willmann, R. *et al. Arabidopsis* lysin-motif proteins LYM1 LYM3 CERK1 mediate bacterial peptidoglycan sensing and immunity to bacterial infection. *Proc. Natl Acad. Sci. USA* **108,** 19824–19829 (2011).
28. Liu, T. *et al.* Chitin-induced dimerization activates a plant immune receptor. *Science* **336,** 1160–1164 (2012).
29. Kouzai, Y. *et al.* Targeted gene disruption of OsCERK1 reveals its indispensable role in chitin perception and involvement in the peptidoglycan response and immunity in rice. *Mol. Plant Microbe Interact.* **27,** 975–982 (2014).
30. Ao, Y. *et al.* OsCERK1 and OsRLCK176 play important roles in peptidoglycan and chitin signaling in rice innate immunity. *Plant J.* **80,** 1072–1084 (2014).
31. Czaja, L. F. *et al.* Transcriptional responses toward diffusible signals from symbiotic microbes reveal MtNFP- and MtDMI3-dependent reprogramming of host gene expression by arbuscular mycorrhizal fungal lipochitooligosaccharides. *Plant Physiol.* **159,** 1671–1685 (2012).
32. Amor, B. B. *et al.* The NFP locus of *Medicago truncatula* controls an early step of Nod factor signal transduction upstream of a rapid calcium flux and root hair deformation. *Plant J.* **34,** 495–506 (2003).
33. Radutoiu, S. *et al.* Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. *Nature* **425,** 585–592 (2003).
34. Zhang, X. *et al.* The receptor kinase CERK1 has dual functions in symbiosis and immunity signalling. *Plant J.* **81,** 258–267 (2015).

35. Op den Camp, R. *et al.* LysM-type mycorrhizal receptor recruited for *Rhizobium* symbiosis in nonlegume *Parasponia*. *Science* **331,** 909–912 (2011).
36. Buendia, L., Wang, T., Girardin, A. & Lefebvre, B. The LysM receptor-like kinase SILYK10 regulates the arbuscular mycorrhizal symbiosis in tomato. *New Phytol.* **210,** 184–195 (2016).
This paper demonstrates that LysM-receptor kinases are required for the association of plants with arbuscular mycorrhizal fungi.
37. Miyata, K. *et al.* Evaluation of the role of the LysM receptor-like kinase, OsNFR5/OsRLK2 for AM symbiosis in rice. *Plant Cell Physiol.* **57,** 2283–2290 (2016).
38. Gaude, N., Bortfeld, S., Duensing, N., Lohse, M. & Krajinski, F. Arbuscule-containing and non-colonized cortical cells of mycorrhizal roots undergo extensive and specific reprogramming during arbuscular mycorrhizal development. *Plant J.* **69,** 510–528 (2012).
39. Gomez, S. K. & Harrison, M. J. Laser microdissection and its application to analyze gene expression in arbuscular mycorrhizal symbiosis. *Pest Manag. Sci.* **65,** 504–511 (2009).
40. Hogekamp, C. *et al.* Laser microdissection unravels cell-type-specific transcription in arbuscular mycorrhizal roots, including CAAT-box transcription factor gene expression correlating with fungal contact and spread. *Plant Physiol.* **157,** 2023–2043 (2011).
41. Fliegmann, J. *et al.* Lipo-chitooligosaccharidic symbiotic signals are recognized by LysM receptor-like kinase LYR3 in the legume *Medicago truncatula*. *ACS Chem. Biol.* **8,** 1900–1906 (2013).
42. Fliegmann, J. *et al.* LYR3, a high-affinity LCO-binding protein of *Medicago truncatula*, interacts with LYK3, a key symbiotic receptor. *FEBS Lett.* **590,** 1477–1487 (2016).
43. Malkov, N. *et al.* Molecular basis of lipo-chitooligosaccharide recognition by the lysin motif receptor-like kinase LYR3 in legumes. *Biochem. J.* **473,** 1369–1378 (2016).
44. De Mita, S., Streng, A., Bisseling, T. & Geurts, R. Evolution of a symbiotic receptor through gene duplications in the legume–*Rhizobium* mutualism. *New Phytol.* **201,** 961–972 (2014).
45. Miyata, K. *et al.* The bifunctional plant receptor, OsCERK1, regulates both chitin-triggered immunity and arbuscular mycorrhizal symbiosis in rice. *Plant Cell Physiol.* **55,** 1864–1872 (2014).
One of several papers that show that CERK1 in rice has a dual role in immunity and symbiosis signalling.
46. Nakagawa, T. *et al.* From defense to symbiosis: limited alterations in the kinase domain of LysM receptor-like kinases are crucial for evolution of legume–*Rhizobium* symbiosis. *Plant J.* **65,** 169–180 (2011).
47. Broghammer, A. *et al.* Legume receptors perceive the rhizobial lipochitin oligosaccharide signal molecules by direct binding. *Proc. Natl Acad. Sci. USA* **109,** 13859–13864 (2012).
48. Madsen, E. B. *et al.* Autophosphorylation is essential for the *in vivo* function of the *Lotus japonicus* Nod factor receptor 1 and receptor-mediated signalling in cooperation with Nod factor receptor 5. *Plant J.* **65,** 404–417 (2011).
49. Pietraszewska-Bogiel, A. *et al.* Interaction of *Medicago truncatula* lysin motif receptor-like kinases, NFP and LYK3, produced in *Nicotiana benthamiana* induces defence-like responses. *PLoS ONE* **8,** e65055 (2013).
50. Ben, C. *et al.* Natural diversity in the model legume *Medicago truncatula* allows identifying distinct genetic mechanisms conferring partial resistance to *Verticillium* wilt. *J. Exp. Bot.* **64,** 317–332 (2013).
51. Rey, T. *et al.* NFP, a LysM protein controlling Nod factor perception, also intervenes in *Medicago truncatula* resistance to pathogens. *New Phytol.* **198,** 875–886 (2013).
52. Rey, T., Chatterjee, A., Buttay, M., Toulotte, J. & Schornack, S. *Medicago truncatula* symbiosis mutants affected in the interaction with a biotrophic root pathogen. *New Phytol.* **206,** 497–500 (2015).
53. Wang, E. *et al.* A common signaling process that promotes mycorrhizal and oomycete colonization of plants. *Curr. Biol.* **22,** 2242–2246 (2012).
54. Downie, J. A. The roles of extracellular proteins, polysaccharides and signals in the interactions of rhizobia with legume roots. *FEMS Microbiol. Rev.* **34,** 150–170 (2010).
55. Gully, D. *et al.* A peptidoglycan-remodeling enzyme is critical for bacteroid differentiation in *Bradyrhizobium* spp. during legume symbiosis. *Mol. Plant Microbe Interact.* **29,** 447–457 (2016).
56. Kawaharada, Y. *et al.* Receptor-mediated exopolysaccharide perception controls bacterial infection. *Nature* **523,** 308–312 (2015).
The first paper to describe the receptors in plants that are responsible for the recognition of exopolysaccharides from bacteria.
57. Cardenas, L. *et al. Rhizobium* Nod factors induce increases in intracellular free calcium and extracellular calcium influxes in bean root hairs. *Plant J.* **19,** 347–352 (1999).
58. Shaw, S. L. & Long, S. R. Nod factor elicits two separable calcium responses in *Medicago truncatula* root hair cells. *Plant Physiol.* **131**, 976–984 (2003).
59. Ma, X., Xu, G., He, P. & Shan, L. SERKing coreceptors for receptors. *Trends Plant Sci.* **21,** 1017–1033 (2016).
60. Halter, T. *et al.* The leucine-rich repeat receptor kinase BIR2 is a negative regulator of BAK1 in plant immunity. *Curr. Biol.* **24,** 134–143 (2014).
61. Yeh, Y. H. *et al.* The *Arabidopsis* malectin-like/LRR-RLK IOS1 is critical for BAK1-dependent and BAK1-independent pattern-triggered immunity. *Plant Cell* **28,** 1701–1721 (2016).
62. Le, M. H., Cao, Y., Zhang, X. C. & Stacey, G. LIK1, a CERK1-interacting kinase, regulates plant immune responses in *Arabidopsis*. *PLoS ONE* **9,** e102245 (2014).
63. Endre, G. *et al.* A receptor kinase gene regulating symbiotic nodule development. *Nature* **417,** 962–966 (2002).
64. Stracke, S. *et al.* A plant receptor-like kinase required for both bacterial and fungal symbiosis. *Nature* **417,** 959–962 (2002).
65. Antolín-Llovera, M., Ried, M. K. & Parniske, M. Cleavage of the SYMBIOSIS RECEPTOR-LIKE KINASE ectodomain promotes complex formation with Nod factor receptor 5. *Curr. Biol.* **24,** 422–427 (2014).
66. Ried, M. K., Antolin-Llovera, M. & Parniske, M. Spontaneous symbiotic reprogramming of plant roots triggered by receptor-like kinases. *eLife* **3,** e03891 (2014).
67. Chen, T. *et al.* A MAP kinase kinase interacts with SymRK and regulates nodule organogenesis in *Lotus japonicus*. *Plant Cell* **24,** 823–838 (2012).
68. Venkateshwaran, M. *et al.* A role for the mevalonate pathway in early plant symbiotic signaling. *Proc. Natl Acad. Sci. USA* **112,** 9781–9786 (2015).
69. Akula, M. K. *et al.* Control of the innate immune response by the mevalonate pathway. *Nature Immunol.* **17,** 922–929 (2016).
70. Zhang, J. *et al.* Receptor-like cytoplasmic kinases integrate signaling from multiple plant immune receptors and are targeted by a *Pseudomonas syringae* effector. *Cell Host Microbe* **7,** 290–301 (2010).
71. Lu, D. *et al.* A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity. *Proc. Natl Acad. Sci. USA* **107,** 496–501 (2010).
72. Shinya, T. *et al.* Selective regulation of the chitin-induced defense response by the *Arabidopsis* receptor-like cytoplasmic kinase PBL27. *Plant J.* **79,** 56–66 (2014).
73. Yamaguchi, K. *et al.* A receptor-like cytoplasmic kinase targeted by a plant pathogen effector is directly phosphorylated by the chitin receptor and mediates rice immunity. *Cell Host Microbe* **13,** 347–357 (2013).
74. Sreekanta, S. *et al.* The receptor-like cytoplasmic kinase PCRK1 contributes to pattern-triggered immunity against *Pseudomonas syringae* in *Arabidopsis thaliana*. *New Phytol.* **207,** 78–90 (2015).
75. Kadota, Y. *et al.* Direct regulation of the NADPH Oxidase RBOHD by the PRR-associated kinase BIK1 during plant immunity. *Mol. Cell* **54,** 43–55 (2014).
76. Li, L. *et al.* The FLS2-associated kinase BIK1 directly phosphorylates the NADPH oxidase RbohD to control plant immunity. *Cell Host Microbe* **15,** 329–338 (2014).
77. Feng, F. *et al.* A *Xanthomonas* uridine 5'-monophosphate transferase inhibits plant immune kinases. *Nature* **485,** 114–118 (2012).
78. Yamada, K. *et al.* The *Arabidopsis* CERK1-associated kinase PBL27 connects chitin perception to MAPK activation. *EMBO J.* **35,** 2468–2483 (2016).
A study that links an activated PAMP receptor complex to MAP kinase activation in response to chitin perception.
79. Andrio, E. *et al.* Hydrogen peroxide-regulated genes in the *Medicago truncatula*–*Sinorhizobium meliloti* symbiosis. *New Phytol.* **198,** 179–189 (2013).
80. Charpentier, M. *et al.* Nuclear-localized cyclic nucleotide-gated channels mediate symbiotic calcium oscillations. *Science* **352,** 1102–1105 (2016).
81. Charpentier, M. *et al. Lotus japonicus* CASTOR and POLLUX are ion channels essential for perinuclear calcium spiking in legume root endosymbiosis. *Plant Cell* **20,** 3467–3479 (2008).
82. Capoen, W. *et al.* Nuclear membranes control symbiotic calcium signaling of legumes. *Proc. Natl Acad. Sci. USA* **108,** 14348–14353 (2011).
83. Lévy, J. *et al.* A putative Ca^{2+} and calmodulin-dependent protein kinase required for bacterial and fungal symbioses. *Science* **303,** 1361–1364 (2004).
84. Mitra, R. M. *et al.* A Ca^{2+}/calmodulin-dependent protein kinase required for symbiotic nodule development: gene identification by transcript-based cloning. *Proc. Natl Acad. Sci. USA* **101,** 4701–4705 (2004).
85. Miller, J. B. *et al.* Calcium/calmodulin-dependent protein kinase is negatively and positively regulated by calcium, providing a mechanism for decoding calcium responses during symbiosis signaling. *Plant Cell* **25,** 5053–5066 (2013).
86. Takeda, N., Maekawa, T. & Hayashi, M. Nuclear-localized and deregulated calcium- and calmodulin-dependent protein kinase activates rhizobial and mycorrhizal responses in *Lotus japonicus*. *Plant Cell* **24,** 810–822 (2012).
87. Gleason, C. *et al.* Nodulation independent of rhizobia induced by a calcium-activated kinase lacking autoinhibition. *Nature* **441,** 1149–1152 (2006).
88. Tirichine, L. *et al.* Deregulation of a Ca^{2+}/calmodulin-dependent kinase leads to spontaneous nodule development. *Nature* **441,** 1153–1156 (2006).
89. Messinese, E. *et al.* A novel nuclear protein interacts with the symbiotic DMI3 calcium and calmodulin dependent protein kinase of *Medicago truncatula*. *Mol. Plant Microbe Interact.* **20,** 912–921 (2007).
90. Yano, K. *et al.* CYCLOPS, a mediator of symbiotic intracellular accommodation. *Proc. Natl Acad. Sci. USA* **105,** 20540–20545 (2008).
91. Singh, S., Katzer, K., Lambert, J., Cerri, M. & Parniske, M. CYCLOPS, a DNA-binding transcriptional activator, orchestrates symbiotic root nodule development. *Cell Host Microbe* **15,** 139–152 (2014).
92. Fonouni-Farde, C. *et al.* DELLA-mediated gibberellin signalling regulates Nod factor signalling and rhizobial infection. *Nature Commun.* **7,** 12636 (2016).
93. Jin, Y. *et al.* DELLA proteins are common components of symbiotic rhizobial and mycorrhizal signalling pathways. *Nature Commun.* **7,** 12433 (2016).
94. Pimprikar, P. *et al.* A CCaMK–CYCLOPS–DELLA complex activates transcription of RAM1 to regulate arbuscule branching. *Curr. Biol.* **26,** 987–998 (2016).
A report that characterizes the calcium-decoding transcription-factor complex that regulates arbuscular mycorrhizal gene expression.
95. Floss, D. S., Levy, J. G., Levesque-Tremblay, V., Pumplin, N. & Harrison, M. J. DELLA proteins regulate arbuscule formation in arbuscular mycorrhizal symbiosis. *Proc. Natl Acad. Sci. USA* **110,** E5025–E5034 (2013).
96. Maekawa, T. *et al.* Gibberellin controls the nodulation signaling pathway in *Lotus japonicus*. *Plant J.* **58,** 183–194 (2009).
97. Navarro, L. *et al.* DELLAs control plant immune responses by modulating the

balance of jasmonic acid and salicylic acid signaling. *Curr. Biol.* **18,** 650–655 (2008).
98. Cárdenas, L., Martinez, A., Sanchez, F. & Quinto, C. Fast, transient and specific intracellular ROS changes in living root hair cells responding to Nod factors (NFs). *Plant J.* **56,** 802–813 (2008).
99. Marino, D. *et al.* A *Medicago truncatula* NADPH oxidase is involved in symbiotic nodule functioning. *New Phytol.* **189,** 580–592 (2011).
100. Montiel, J. *et al.* A *Phaseolus vulgaris* NADPH oxidase gene is required for root infection by rhizobia. *Plant Cell Physiol.* **53,** 1751–1767 (2012).
101. Morieri, G. *et al.* Host-specific Nod-factors associated with *Medicago truncatula* nodule infection differentially induce calcium influx and calcium spiking in root hairs. *New Phytol.* **200,** 656–662 (2013).
102. Garcia-Brugger, A. *et al.* Early signaling events induced by elicitors of plant defenses. *Mol. Plant Microbe Interact.* **19**, 711–724 (2006).
103. Nars, A. *et al. Aphanomyces euteiches* cell wall fractions containing novel glucan-chitosaccharides induce defense genes and nuclear calcium oscillations in the plant host *Medicago truncatula*. *PLoS ONE* **8,** e75039 (2013).
104. Thor, K. & Peiter, E. Cytosolic calcium signals elicited by the pathogen-associated molecular pattern flg22 in stomatal guard cells are of an oscillatory nature. *New Phytol.* **204,** 873–881 (2014).
Refs 104 and 105 use live single-cell imaging to reveal that PAMP perception induces cytosolic oscillatory calcium signals.
105. Keinath, N. F. *et al.* Live cell imaging with R-GECO1 sheds light on flg22- and chitin-induced transient $[Ca^{2+}]_{cyt}$ patterns in *Arabidopsis*. *Mol. Plant* **8,** 1188–1200 (2015).
106. Seybold, H. *et al.* Ca^{2+} signalling in plant immune response: from pattern recognition receptors to Ca^{2+} decoding mechanisms. *New Phytol.* **204,** 782–790 (2014).
107. Yu, F., Tian, W. & Luan, S. From receptor-like kinases to calcium spikes: what are the missing links? *Mol. Plant* **7,** 1501–1504 (2014).
108. Boudsocq, M. *et al.* Differential innate immune signalling via Ca^{2+} sensor protein kinases. *Nature* **464,** 418–422 (2010).
109. Dubiella, U. *et al.* Calcium-dependent protein kinase/NADPH oxidase activation circuit is required for rapid defense signal propagation. *Proc. Natl Acad. Sci. USA* **110,** 8744–8749 (2013).
110. Ranf, S., Eschen-Lippold, L., Pecher, P., Lee, J. & Scheel, D. Interplay between calcium signalling and early signalling elements during defence responses to microbe- or damage-associated molecular patterns. *Plant J.* **68,** 100–113 (2011).
111. Evans, M. J., Choi, W. G., Gilroy, S. & Morris, R. J. A ROS-assisted calcium wave dependent on the AtRBOHD NADPH oxidase and TPC1 cation channel propagates the systemic response to salt stress. *Plant Physiol.* **171,** 1771–1784 (2016).
112. Gilroy, S. *et al.* ROS, calcium, and electric signals: key mediators of rapid systemic signaling in plants. *Plant Physiol.* **171,** 1606–1615 (2016).
113. Yang, S., Tang, F., Gao, M., Krishnan, H. B. & Zhu, H. *R* gene-controlled host specificity in the legume–rhizobia symbiosis. *Proc. Natl Acad. Sci. USA* **107,** 18735–18740 (2010).
114. Deakin, W. J. & Broughton, W. J. Symbiotic use of pathogenic strategies: rhizobial protein secretion systems. *Nature Rev. Microbiol.* **7,** 312–320 (2009).
115. Kloppholz, S., Kuhn, H. & Requena, N. A secreted fungal effector of *Glomus intraradices* promotes symbiotic biotrophy. *Curr. Biol.* **21,** 1204–1209 (2011).
116. Marchetti, M. *et al.* Experimental evolution of a plant pathogen into a legume symbiont. *PLoS Biol.* **8,** e1000280 (2010).
117. Okazaki, S., Kaneko, T., Sato, S. & Saeki, K. Hijacking of leguminous nodulation signaling by the rhizobial type III secretion system. *Proc. Natl Acad. Sci. USA* **110,** 17131–17136 (2013).
This paper presents exciting evidence to show that bacterial effectors can activate nodulation signalling without the need for Nod factors.
118. Giraud, E. *et al.* Legume symbioses: absence of *Nod* genes in photosynthetic bradyrhizobia. *Science* **316,** 1307–1312 (2007).
119. Gourion, B., Berrabah, F., Ratet, P. & Stacey, G. *Rhizobium*–legume symbioses: the crucial role of plant immunity. *Trends Plant Sci.* **20,** 186–194 (2015).
120. Liang, Y. *et al.* Nonlegumes respond to rhizobial Nod factors by suppressing the innate immune response. *Science* **341,** 1384–1387 (2013).
This paper shows that LCOs can suppress immunity signalling in a wide array of plant species.
121. Lebeis, S. L. *et al.* Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science* **349,** 860–864 (2015).
This paper shows the importance of defence signalling for regulating bacterial associations in the plant microbiome.
122. Berrabah, F. *et al.* A nonRD receptor-like kinase prevents nodule early senescence and defense-like reactions during symbiosis. *New Phytol.* **203,** 1305–1314 (2014).
123. Berrabah, F., Ratet, P. & Gourion, B. Multiple steps control immunity during the intracellular accommodation of rhizobia. *J. Exp. Bot.* **66,** 1977–1985 (2015).
This paper demonstrates that a suite of genes are required to regulate immunity in the nitrogen-fixing nodule.
124. Domonkos, A. *et al.* The identification of novel loci required for appropriate nodule development in *Medicago truncatula*. *BMC Plant Biol.* **13,** 157 (2013).
125. Wang, C. *et al. NODULES WITH ACTIVATED DEFENSE 1* is required for maintenance of rhizobial endosymbiosis in *Medicago truncatula*. *New Phytol.* **212,** 176–191 (2016).
126. Bourcy, M. *et al. Medicago truncatula* DNF2 is a PI–PLC–XD-containing protein required for bacteroid persistence and prevention of nodule early senescence and defense-like reactions. *New Phytol.* **197,** 1250–1261 (2013).
127. Zipfel, C. Plant pattern-recognition receptors. *Trends Immunol.* **35,** 345–351 (2014).
128. Zuo, W. *et al.* A maize wall-associated kinase confers quantitative resistance to head smut. *Nature Genet.* **47,** 151–157 (2015).
129. Liu, Y. *et al.* A gene cluster encoding lectin receptor kinases confers broad-spectrum and durable insect resistance in rice. *Nature Biotechnol.* **33,** 301–305 (2015).
130. Hurni, S. *et al.* The maize disease resistance gene *Htn1* against northern corn leaf blight encodes a wall-associated receptor-like kinase. *Proc. Natl Acad. Sci. USA* **112,** 8780–8785 (2015).
131. Hind, S. R. *et al.* Tomato receptor FLAGELLIN-SENSING 3 binds flgII-28 and activates the plant immune system. *Nature Plants* **2,** 16128 (2016).
132. Shiu, S. H. & Bleecker, A. B. Expansion of the *receptor-like kinase/Pelle* gene family and receptor-like proteins in *Arabidopsis*. *Plant Physiol.* **132,** 530–543 (2003).
133. Fischer, I., Dievart, A., Droc, G., Dufayard, J. F. & Chantret, N. Evolutionary dynamics of the leucine-rich repeat receptor-like kinase (LRR-RLK) subfamily in angiosperms. *Plant Physiol.* **170,** 1595–1610 (2016).
134. Lacombe, S. *et al.* Interfamily transfer of a plant pattern-recognition receptor confers broad-spectrum bacterial resistance. *Nature Biotechnol.* **28,** 365–369 (2010).
135. Mendes, B. M. J. *et al.* Reduction in susceptibility to *Xanthomonas axonopodis* pv. *citri* in transgenic *Citrus sinens*is expressing the rice *Xa21* gene. *Plant Pathol.* **59,** 68–75 (2010).
136. Tripathi, J. N., Lorenzen, J., Bahar, O., Ronald, P. & Tripathi, L. Transgenic expression of the rice Xa21 pattern-recognition receptor in banana (*Musa* sp.) confers resistance to *Xanthomonas campestris* pv. *musacearum*. *Plant Biotechnol. J.* (2014).
137. Schoonbeek, H.-J. *et al. Arabidopsis* EF-Tu receptor enhances bacterial disease resistance in transgenic wheat. *New Phytol.* **206,** 606–613 (2015).
138. Schwessinger, B. *et al.* Transgenic expression of the dicotyledonous pattern recognition receptor EFR in rice leads to ligand-dependent activation of defense responses. *PLoS Pathog.* **11,** e1004809 (2015).
139. Lu, F. *et al.* Enhancement of innate immune system in monocot rice by transferring the dicotyledonous elongation factor Tu receptor EFR. *J. Integr. Plant Biol.* **57,** 641–652 (2015).
140. Du, J. *et al.* Elicitin recognition confers enhanced resistance to *Phytophthora infestans* in potato. *Nature Plants* **1,** 15034 (2015).
141. Albert, I. *et al.* An RLP23–SOBIR1–BAK1 complex mediates NLP-triggered immunity. *Nature Plants* **1,** 15140 (2015).
142. Hao, G., Pitino, M., Duan, Y. & Stover, E. Reduced susceptibility to *Xanthomonas citri* in transgenic citrus expressing the FLS2 receptor from *Nicotiana benthamiana*. *Mol. Plant Microbe Interact.* **29,** 132–142 (2016).
143. Stokstad, E. The nitrogen fix. *Science* **353,** 1225–1227 (2016).
144. Ivleva, N. B., Groat, J., Staub, J. M. & Stephens, M. Expression of active subunit of nitrogenase via integration into plant organelle genome. *PLoS ONE* **11,** e0160951 (2016).
145. López-Torrejón, G. *et al.* Expression of a functional oxygen-labile nitrogenase component in the mitochondrial matrix of aerobically grown yeast. *Nature Commun.* **7,** 11426 (2016).

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