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Microreview

Mutualism versus pathogenesis: the give-and-take in plant–bacteria interactions

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Summary

Pathogenic bacteria and mutualistic rhizobia are able to invade and establish chronic infections within their host plants. The success of these plant–bacteria interactions requires evasion of the plant innate immunity by either avoiding recognition or by suppressing host defences. The primary plant innate immunity is triggered upon recognition of common microbe-associated molecular patterns. Different studies reveal striking similarities between the molecular bases underlying the perception of rhizobial nodulation factors and microbe-associated molecular patterns from plant pathogens. However, in contrast to general elicitors, nodulation factors can control plant defences when recognized by their cognate legumes. Nevertheless, in response to rhizobial infection, legumes show transient or local defence-like responses suggesting that *Rhizobium* is perceived as an intruder although the plant immunity is controlled. Whether these responses are involved in limiting the number of infections or whether they are required for the progression of the interaction is not yet clear. Further similarities in both plant–pathogen and *Rhizobium*–legume associations are factors such as surface polysaccharides, quorum sensing signals and secreted proteins, which play important roles in modulating plant defence responses and determining the outcome of the interactions.

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Introduction

Plants represent an important source of water and nutrients for microbes. To get access to these nutrients, several pathogenic and mutualistic bacteria such as rhizobia are able to colonize, invade and establish chronic infections within their plant hosts albeit leading to different outcomes. Whereas phytopathogenic bacteria cause damage and often impair plant growth and reproduction, infection of legumes by rhizobia results in an overall benefit based on nutrient exchange. During the legume–rhizobia interaction, the bacteria invade the plant root, leading to the formation of a specialized organ, the nodule, where they reduce nitrogen into ammonia that can be used by the plant, allowing legumes to grow in nitrogen-depleted soils.

During the infection process, phytopathogenic bacteria enter plant tissues either by wounds or natural openings and occupy the apoplast of plant tissues or the xylem where they multiply and spread, a process that often involves the participation of hydrolytic enzymes and toxins. On the other hand, the formation of nitrogen-fixing nodules is a more complex process in which rhizobial infection needs to be co-ordinated with a root developmental program. This multistep process requires the mutual secretion and correct recognition of several signal molecules by both the plant and bacteria (reviewed in Oldroyd and Downie, 2008). Flavonoids excreted by the plant specifically induce in the bacterium the production of the Nod factor (NF), a lipo-chito-oligosaccharide nodulation signal. NFs induce several responses in the plant such as curling of the root hairs and the formation of nodule primordia after the activation of cortical cell division. Bacteria attached to root hairs penetrate the root through a tubular structure called the infection thread, which grows towards the root cortex where the nodule primordium is developing. When the thread reaches the primordium, the bacteria are released into the plant cytoplasm where they differentiate into their endosymbiotic form, the bacteroids.

Whereas it is clear that in plant–pathogen interactions the host tries to restrict growth and reproduction of the invading microorganism, in the rhizobia–legume symbiosis no obvious plant defence responses appear to be involved. A question raised for a long time is whether

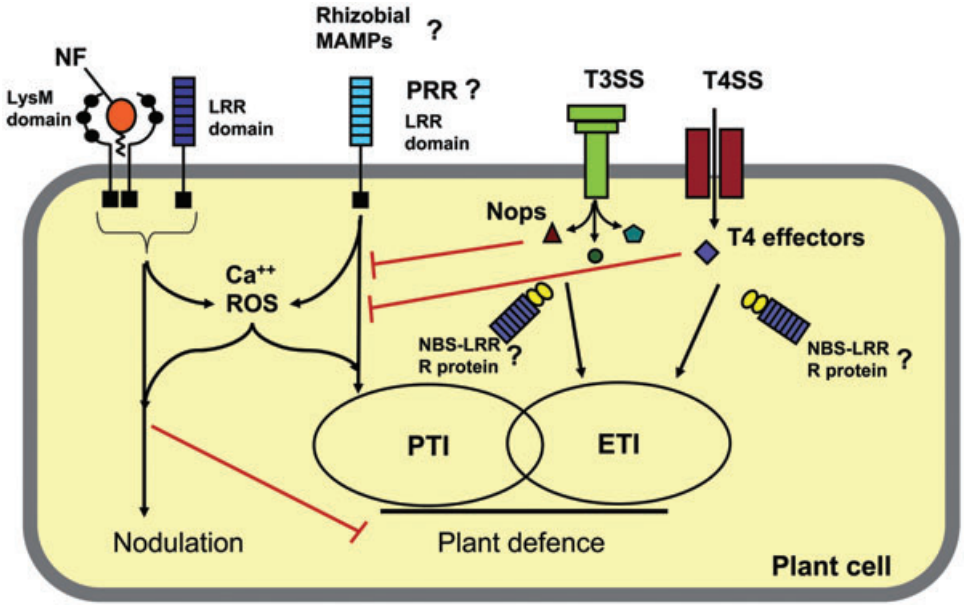

Fig. 1. Model depicting plant responses upon recognition of different rhizobial components based on the knowledge available on plant–pathogen interactions. Plants are able to recognize PAMPs or MAMPs through cell-surface PRR activating plant defences known as PTI. Usually PRRs contain extracellular LRRs and intracellular kinase domains (LRR-RLK). Although there is evidence for the existence of general elicitors in *Rhizobium* (see text for details), the identity of rhizobial MAMPs and the cognate plant PRRs remain unknown. NF perception involves LysM-containing RLKs and an LRR-RLK. Early events upon recognition of both PAMPs and NF include changes in calcium levels and the production of ROS. In contrast to recognition of PAMPs, NF perception by its cognate legume host suppresses some plant defences and promotes bacterial infection and nodule organogenesis. Effector proteins translocated through the action of T3SS help bacterial pathogens to suppress PTI. Likewise, type III effectors identified in several rhizobia (Nops) and T4 effectors identified in *Mesorhizobium loti* could play similar roles acting in a host-specific manner. To survive to pathogen attack, plants have evolved intracellular resistance (R) proteins usually containing nucleotide-binding site and LRR domains (NB-LRR) that are able to recognize the action of bacterial effectors and trigger an additional line of defence known as ETI. The nature of rhizobial-specific R proteins in legumes is unknown.

plants are able to discriminate between pathogenic bacteria and rhizobia. Accumulating evidence indicates that both plant pathogens and rhizobia are perceived as intruders by the host plant triggering similar responses, with the success of the interaction depending mostly on the ability of the bacteria to either block and/or overcome the defences mounted by the plant. This review focuses on recent advances accounting for similarities found in plant responses during pathogenic and symbiotic interactions.

Early perception of rhizobia and pathogenic bacteria in legume and non-legume plants

Plants rely on innate immunity to defend against microbial invaders (Chisholm *et al*., 2006; Jones and Dangl, 2006). Studies performed in plant–pathogen interactions indicate that the first line of plant defence is triggered upon the recognition of general elicitors, known as microbe-associated (or pathogen-associated) molecular patterns (MAMPs/PAMPs), by plant transmembrane pattern-recognition receptors (PRR). This recognition results in the so-called PAMP-triggered immunity (PTI) or basal defence that negatively affects the progress of bacterial infection before the microbe gains a hold in the plant. Different studies reveal striking similarities between initial perception of *Rhizobium* and the molecular bases of PTI triggered by plant pathogens (Fig. 1).

The MAMPs are epitopes within molecules that are essential for microbial life, absent in the host and widely distributed among different microorganisms (Zipfel, 2008). For obvious reasons, studies dealing with MAMP identification and their perception by plants have been mainly performed with phytopathogenic bacteria. These studies have revealed that plants have evolved perception systems for different bacterial MAMPs like flagellin, lipopolysaccharide (LPS), elongation factor Tu (EF-Tu), cold shock protein (CSP) or peptidoglycan (PGN) (He *et al*., 2007; Erbs *et al*., 2008). MAMP perception triggers several plant responses. Early reactions, occurring within minutes, include ion-flux across the plasma membrane, increased intracellular Ca^{2+} concentration, oxidative burst, MAP kinase activation and major transcriptional changes (Nürnberger *et al*., 2004). Interestingly, many of these responses have also been detected in epidermal cells of legume roots soon after application of NFs (Felle *et al*., 2000; Ramu *et al*., 2002). Thus, the initial responses of legume plants to the symbiotic signal resemble those triggered by MAMPs with the difference being that strong plant defences are not mounted. In fact, we and others have proposed that NFs could play a role in the control of

plant defence reactions, suppressing salicylic acid (SA) accumulation and reactive oxygen species (ROS) production in the cognate legume host (Martínez-Abarca *et al*., 1998; Bueno *et al*., 2001; Shaw and Long, 2003). On the contrary, the application of NFs induces a typical defence reaction in a non-legume plant (Staehelin *et al*., 1994). Like NFs, rhizobial LPSs have been proposed to act as specific suppressors of plant defence responses in their cognate legume, while eliciting defence reactions in non-legume plants (Albus *et al*., 2001; Scheidle *et al*., 2005; Tellström *et al*., 2007). It is possible, as it has been already proposed (Hirsch, 2004; Tellström *et al*., 2007), that legume plants harbour different perception systems for certain bacterial components such as LPS or chitooligosaccharides-related molecules, allowing discrimination between pathogenic bacteria and rhizobia.

Most plant PRRs are receptor-like kinases (RLKs) or receptor-like proteins. Two of the most studied PRRs are FLS2 and EFR, leucine-rich repeat (LRR)-RLKs that recognize flagellin and EF-Tu respectively (Chinchilla *et al*., 2006; Zipfel *et al*., 2006). In legumes, NF perception involves an LRR-RLK (Endre *et al*., 2002) and lysine motifs (LysM)-containing RLKs, the latter being excellent candidates as NF receptors (Limpens *et al*., 2003; Madsen *et al*., 2003; Radutoiu *et al*., 2003). Interestingly, a protein recently identified as critical for chitin signalling in *Arabidopsis*, is also a LysM RLK (Wan *et al*., 2008). The structural similarities between chitin and NF and the fact that LysM RLK family members are required for both molecules signalling suggest an evolutionary relationship between the two pathways.

Recent research on MAMP perception has revealed several important points that demonstrate a diversity of PRR specificities (Schwessinger and Zipfel, 2008): (i) some MAMPs are only perceived by certain plant species, (ii) MAMPs are more variable than initially presumed and (iii) the recognized epitope for a given MAMP can vary between plant species. Thus, while most bacterial flagellins are recognized by a wide array of plant species, rhizobial flagellins are inactive as elicitors in *Arabidopsis* (Gómez-Gómez *et al*., 1999), and while rhizobial EF-Tu is recognized as a MAMP in *Arabidopsis*, it does not elicit defence responses in legume plants (Kunze *et al*., 2004). These findings highlight the interest of investigating PTI in *Rhizobium*–plant interactions, specially the identification of possible rhizobial elicitors recognized by host and non-host legume species, their cognate PRRs and the study of their importance in the symbiotic interaction.

Plant defence responses during pathogenic and symbiotic interactions

In addition to PTI, plants have evolved a second line of defence acting largely inside the cell known as effector-triggered immunity (ETI) in which nucleotide-binding site-LRR resistance proteins (R proteins) 'guard' specific microbial effector-mediated perturbations of host cell functions (Jones and Dangl, 2006). Although transcriptomic analyses have revealed a significant overlap between PAMP- and ETI-responsive genes, ETI is quantitatively stronger than PTI and usually results in a hypersensitive cell death response (HR) at the infection site (Schwessinger and Zipfel, 2008). Plant defence-like responses have also been detected in the *Rhizobium*–legume interaction, such as features characteristic of the HR and the presence of molecules like SA, ROS and nitric oxide (NO). The role for some of these responses in symbiosis is not yet clear.

The HR is one of the most obvious plant defence mechanisms against avirulent pathogens with the characteristic appearance of necrotic flecks containing dead plant cells at sites of attempted microorganism ingress (Heath, 2000). Very localized hypersensitive-like reactions have been reported to occur in the *Sinorhizobium meliloti*–alfalfa interaction, associated to aborted infection threads in the epidermis or cortex. It has been proposed that this reaction may be part of the mechanism mediating the plant host's autoregulation of nodule number (Vasse *et al*., 1993). Local cell death has also been detected during the intercellular colonization required for lateral root base nodulation of the semiaquatic legume *Sesbania rostrata* (D'Haeze *et al*., 2003). In this case, dead cells could fortify aerenchymatic tissues as part of the water stress response.

The SA is a phenolic metabolite that plays key roles in plant disease resistance. This molecule is intimately involved in both the localized HR and systemic acquired resistance (Durner *et al*., 1997), and can also interfere directly with several aspects of the infection process of pathogenic bacteria (Yuan *et al*., 2007). To our knowledge, no SA accumulation has been reported to occur in legumes upon recognition of a compatible *Rhizobium*. However, evidence exists that SA plays a role in the establishment of symbiosis. Increases in root-SA levels in alfalfa plants have been observed after inoculation with rhizobia unable to produce NFs, and the application of exogenous SA delays and inhibits the formation of indeterminate nodules (Martínez-Abarca *et al*., 1998; van Spronsen *et al*., 2003). The role for SA-mediated plant defence pathways in controlling nodule formation in legumes has been more recently supported by the observation that reduced endogenous SA levels caused by the transgenic expression of salicylate hydroxylase (NahG) in two model legumes leads to enhanced nodulation and infection (Stacey *et al*., 2006).

Plant perception of avirulent pathogens leads to a biphasic oxidative burst: a transient and non-specific weak oxidative burst followed by a massive production of

ROS (Lamb and Dixon, 1997). A number of possible functions for ROS in plant defence have been proposed: direct killing of pathogens, involvement in structural changes to reinforce the cell wall, promotion of the HR and induction of defence gene expression. On the contrary, the role of ROS accumulation observed in several situations of the *Rhizobium*–legume interaction is not clear. An intense oxidative burst could be interpreted as a defence response aimed to control and limit bacterial entry. This could be the case for ROS detected in aborted infection threads, or the higher H_2O_2 accumulation observed in plants inoculated with a *Rhizobium* unable to nodulate (Vasse *et al*., 1993; Bueno *et al*., 2001). On the other hand, the prolonged and localized production of ROS observed in infection threads and in infected plant cells, together with the requirement of ROS accumulation for the expression of an early nodulin (Rip1) in *Medicago truncatula* and for nodule initiation in *S. rostrata*, suggests that the oxidative burst might have a positive role in the establishment of the symbiotic interaction (Hérouart *et al*., 2002; Ramu *et al*., 2002; D'Haeze *et al*., 2003). ROS might be necessary for an adequate progression of the infection threads participating in modifications of the plant cell wall or in the insolubilization of the infection thread matrix. Not excluded is the possibility that a critical concentration of ROS could be a signal for the expression of plant and/or bacterial symbiotic genes.

Concomitant with the production of ROS, the recognition of an avirulent pathogen by a resistant plant induces a rapid accumulation of NO. NO has been shown to trigger HR, activate the expression of several defence genes, increase the level of ROS within the cell and modulate the synthesis of defence-related compounds such as SA, jasmonic acid and ethylene (Delledonne, 2005). Different reports also point to a possible role for NO in the *Rhizobium*–legume symbiosis. Thus, NO production has been detected in nitrogen-fixing nodules (Baudouin *et al*., 2006) and modulation of the levels of NO leads to the modification of the number of indeterminate nodules per root (Pii *et al*., 2007). More recently, the differential behaviour of NO-responsive genes observed in *M. truncatula* in response to pathogenic or symbiotic interactions suggests a possible role for this molecule as a transcriptional modulator in different plant–microbe interactions (Ferrarini *et al*., 2008).

Polysaccharides and quorum sensing signals: bacterial factors interfering with plant defence responses

The establishment of a compatible plant–bacteria association, regardless of the bacteria being pathogenic or *Rhizobium*, requires either evading detection or, failing that, avoiding plant defences that could abort microbial infection. It is well known that successful pathogens are able to evade plant innate immunity by using different strategies (Abramovitch *et al*., 2006; Soto *et al*., 2006; López *et al*., 2008; Navarro *et al*., 2008). In the case of the *Rhizobium*–legume symbiosis, most plant defence-like responses detected are transient and local, and recent transcriptomic analyses have revealed the repression of defence-associated genes during nodule development, suggesting the control of plant immunity (El Yahyaoui *et al*., 2004; Kouchi *et al*., 2004; Brechenmacher *et al*., 2008). How plant-associated bacteria are able to overcome and suppress the host defences is the subject of active research. The role in this process of effector proteins secreted by specialized transporters has been studied extensively and will be discussed in the next section. However, growing evidence points to additional bacterial components as players in the control of plant defences.

Bacterial surface polysaccharides (SPSs) are crucial in the establishment of plant–bacteria interactions. Rhizobial mutants altered in production of exopolysaccharides, LPSs or cyclic β-glucans are usually defective in nodule invasion. Likewise, plant pathogenic bacteria defective in SPSs have altered virulence. As with plant pathogens, it is believed that SPSs protect rhizobia acting as physical barriers for defence compounds released by the host during invasion, such as ROSs or other antimicrobial compounds (D'Haeze and Holsters, 2004). But SPSs can also act as suppresors of host defence responses. For instance, cyclic β-glucans from *Bradyrhizobium japonicum* can suppress defence responses induced by the β-heptaglucoside elicitor from *Phytophtora sojae* (Mithöfer *et al*., 1996). Related to this, cyclic β-glucans from the pathogen *Xanthomonas campestris* have a role in systemic suppression of plant defence responses (Rigano *et al*., 2007). Likewise, the lipid A component of *S. meliloti* LPS can suppress the oxidative burst and expression of defence genes in host plant species; in contrast, *S. meliloti* LPS induces an oxidative burst in cultured cells of the non-host tobacco (Scheidle *et al*., 2005; Tellström *et al*., 2007). More recently, various polyanionic SPSs from pathogenic and mutualistic bacteria have been shown to suppress MAMP-induced innate immunity through a mechanism involving chelation of apoplastic Ca^{2+} (Aslam *et al*., 2008). Certainly more studies are needed to fully understand the actual roles of bacterial SPSs in modulating plant defences.

The involvement of quorum sensing (QS) signals and QS-mimics in plant–bacteria communication is an emerging area of research. In response to cell densities, QS signals (or autoinducers) allow bacterial populations to co-ordinate the expression of genes important for the colonization and infection of their hosts. Plant pathogenic bacteria or symbionts defective in QS signalling are

usually defective in invasion of their hosts (von Bodman *et al*., 2003; González and Marketon, 2003). The same bacterial QS signals, however, also participate in cross-kingdom signalling inducing in the plant common and specific changes, which suggest that they may discriminate between QS signals from different bacteria (Bauer and Mathesius, 2004). These signals affect the secretion by roots of proteins, some of them with a role in host defence responses, and QS-mimics that potentially could interfere with QS-regulated behaviours in the bacteria. The structure of plant QS-mimics is yet unknown; however, riboflavin and its derivative lumichrome have recently been identified as putative QS-mimics able to activate the *Pseudomonas aeruginosa* LasR QS receptor (Rajamani *et al*., 2008). How plants can detect and respond to QS signals from rhizobia and pathogenic bacteria, how production of QS-mimics is regulated in the plant and how this may be linked to plant defence responses are questions that need to be answered in the future.

Manipulation of plant responses by bacterial secreted proteins

It is becoming increasingly clear that protein secretion is important in determining the outcome of plant–bacteria interactions. Among the wide variety of proteins exported by plant-associated bacteria, the substrates of type III and type IV secretion systems (T3SS and T4SS respectively) play key roles as they are delivered directly into the host cytoplasm altering host responses.

Proteins secreted by T3SS, or T3 effectors, are crucial for successful pathogenesis mostly due to their ability to suppress plant innate immunity and recent reviews have been published dealing with their effects in plant cells (Grant *et al*., 2006; Block *et al*., 2008). T3SSs with a role in the *Rhizobium*–legume symbiotic process have been identified in several rhizobia. Like in plant–pathogen bacteria, rhizobial T3SS help to modulate the host range and therefore the corresponding effectors may share common functions. Proteins secreted through rhizobial T3SS are called Nodulation Outer Proteins or Nops. Three rhizobial proteins, NopL, NopP and NopT, have been reported as candidates for effectors affecting symbiosis in a specific manner. NopL and NopP are phosphorylated by plant kinases, suggesting that these proteins might affect signal transduction pathways during the establishment of symbiosis with legumes (Bartsev *et al*., 2004; Skorpil *et al*., 2005). Specifically, NopL modulates the activity of signal transduction pathways that culminate in the activation of the plant inducible defence proteins, the so-called pathogenesis-related proteins (Bartsev *et al*., 2004). NopT is a functional cysteine protease of the YopT-AvrPphB family with autoproteolytic activity and a predicted myristoylation site. When transiently expressed in tobacco plants, proteolytically active NopT elicited a rapid HR. Moreover, *Arabidopsis* plants transformed with *nopT* showed chlorotic and necrotic symptoms, indicating a cytotoxic effect (Dai *et al*., 2008). Other candidate effectors include the *Sinorhizobium fredii* NopD and NopM that display similarities with XopD of *Xanthomomas* spp. and YopM of *Yersinia* spp. respectively (Rodrigues *et al*., 2007). XopD and YopM are key for pathogenicity, interfering in the host cell nuclei with the regulation of host proteins during the infection (Skrzypek *et al*., 1998; Hotson *et al*., 2003). Comparative genome analyses will likely identify additional protein effectors conserved among pathogenic bacteria and rhizobia. Nevertheless, an important point to be addressed is which of these putative rhizobial effectors are actually injected into plant cells and more importantly, which cell types are their targets.

On the other hand, T4SS are multiprotein dynamic bacterial surface apparatus specialized in the transfer of (nucleo) protein complexes across cell envelopes. T4SS are essential for bacterial conjugation and for bacterial pathogenicity, such as agrobacteria-induced tumour formation in plant cells (for a review, Christie and Cascales, 2005). A role of T4SS in the conjugative transfer of symbiotic plasmids among rhizobia has been proposed in *Rhizobium etli* (Pérez-Mendoza *et al*., 2005) and *S. meliloti* (Jones *et al*., 2007). Two candidate T4SS effector proteins were identified in *Mesorhizobium loti* strain R7A with an important role in symbiosis: Msi059 and Msi061 that show significant similarities to known T3 and T4 effectors of plant pathogens (Hubber *et al*., 2004). Similar to Nops, their symbiotic role is host-specific, assisting or impeding nodulation depending on the legume species. Msi059 could have a role similar to that of the T3 effector protein XopD of *X. campestris* pv *vesicatoria* shown to exhibit plant-specific SUMO substrate protease activity, whereas Msi061 could act as the VirF of *Agrobacterium tumefaciens*, targeting proteins for ubiquitination and subsequent degradation. The role of T4SS and their putative effectors in symbiosis remains to be clarified as T4SS mutants in other rhizobia like *S. meliloti* did not exhibit apparent symbiotic defects (Jones *et al*., 2007).

Concluding remarks

Legumes and non-legumes seem to have similar perception systems and responses against the attack by any microbe. Both mutualists and pathogenic bacteria are all perceived as intruders by plants and use similar infection weapons and strategies to elude or modulate the plant's battery of resources directed to arrest bacterial progression (Fig. 1). Rhizobia, however, seem to have adapted and refined some of their strategies to interact with

legumes, whereas legumes may have also evolved to discriminate between rhizobia and other microbes, adapting their perception systems and defence responses to the invading microorganism. Rhizobia probably harbour elicitors serving as MAMPs for legumes, whereas rhizobial NFs seem to have evolved to comply with a dual role in plant defence suppression and nodule organogenesis. It is also striking that some compounds, like ROS or NO normally produced by plants to attack the intruders, may have a dual role in the symbiosis, necessary for the progress of the association while toxic for the microsymbiont. Similarly, it is possible that other plant defence mechanisms have evolved in legumes to respond specifically to rhizobia, to allow symbiosis establishment without compromising plant integrity in a refined give-and-take between mutualism and pathogenesis.

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