Symbiosis specificity in the legume – rhizobial mutualism

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Summary

Legume plants are able to engage in root nodule symbiosis with nitrogen-fixing soil bacteria, collectively called rhizobia. This mutualistic association is highly specific, such that each rhizobial species/strain interacts with only a specific group of legumes, and vice versa. Symbiosis specificity can occur at multiple phases of the interaction, ranging from initial bacterial attachment and infection to late nodule development associated with nitrogen fixation. Genetic control of symbiosis specificity is complex, involving fine-tuned signal communication between the symbiotic partners. Here we review our current understanding of the mechanisms used by the host and bacteria to choose their symbiotic partners, with a special focus on the role that the host immunity plays in controlling the specificity of the legume – rhizobial symbiosis.

Introduction

Legumes have the remarkable ability to establish a symbiotic relationship with nitrogen-fixing soil bacteria, known as rhizobia. The mutualism culminates in the formation of a new plant organ, called the root nodule, within which the microsymbionts convert atmospheric nitrogen into ammonia, a biological form that can be directly consumed by the plant. This biological process plays a critical role in sustainable agriculture, because it reduces the need for exogenous nitrogen fertilizer while providing an efficient way of producing protein-rich foods.

Rhizobial infection and nodule development follow a well-defined morphological program. The two partners first establish contact with each other at the surface of the growing tip of a root hair. If the initial dialogue is successful, the root hair curls to trap a small number of bacteria. From this trap site, the root hair begins an inverse tip growth, forming a long and narrow passage, called the infection thread, in which the bacteria ‘travel’ by continuously dividing at the leading edge (Oldroyd et al., 2011). The infection thread traverses the entire length of the cell, eventually merging with the basal membrane to release the bacteria into the extracellular space. Additional infection threads then form in cells of underlying layers, repeating the process to deliver bacteria to their final destination, the root cortex. While the bacteria penetrate the root, host cells in the root cortex reacquire properties of stem cells, giving rise to a population of newly generated cells, which form the lateral organ nodule. When bacteria reach these nodule cells, they are internalized in an endocytosis-like process. Individual ingested bacteria are surrounded by a host membrane, where they differentiate into dedicated nitrogen-fixing organelles named the symbiosome. Fixed nitrogen from the bacteria and fixed carbon provided by the host are traded across the symbiosome membrane.

One striking feature of the legume – rhizobial symbiosis is its high level of specificity. Such specificity can occur both at the early stages of the interaction that are associated with bacterial infection and nodule development as well as at the late stages that are related to nitrogen fixation. Symbiotic specificity has long generated intense interest in the scientific community. From a basic science perspective, specificity in this system is strikingly similar to host – pathogen interactions. Are specificity determinants shared between antagonistic and friendly interactions? If so, how are opposite outcomes achieved? If not, then what are the different features that a host would recognize to distinguish friend from foe? From an applied aspect, understanding the molecular mechanisms underlying symbiotic specificity can lead to improved crop yield.
through better practice, without the need for substantial input. It has been documented that domesticated crop species tend to have fewer compatible symbionts (higher specificity) than their wild counterparts (Mutch and Young, 2004). Such a constraint can lead to decreased yield in soils where the favourable strains are unavailable. On the other hand, even though many legumes can nodulate with indigenous soil bacteria, nitrogen fixation efficiency varies tremendously between different host – rhizobial combinations (Schumpp and Deakin, 2010). Knowledge of genetic control of symbiosis specificity will improve our ability to predict and manipulate key genetic factors controlling the symbiotic interaction and allow researchers to develop new crop varieties or engineer novel rhizobial strains that are able to enhance the agronomic potential of the root nodule symbiosis.

Establishing a successful interaction requires signal recognition between the two symbiotic partners (Fig. 1). Thus, the evolution of host specificity involves both rhizobial and host genes. In this review, we will give a brief overview of our latest understanding of the molecular players from both the host and the microsymbiont that are involved in determining the specificity of the legume – rhizobial symbiosis.

Fig. 1. Molecular determinants of host specificity during nitrogen-fixing symbiosis.

1. At the first stage, the plant produces flavonoid signals (such as the luteolin shown here, from M. truncatula) to free-living soil bacteria, activating the bacterial NodD proteins. NodD proteins bind to the conserved nod-box in the promoters of bacterial nodulation genes to induce their expression.

2. The nod genes code for enzymes for the synthesis of Nod factors. Secreted Nod factors are recognized on the plant surface by transmembrane Nod factor receptors in a strain- and ecotype-specific fashion. Modifications on the Nod factor such as the length and saturation of the acyl group determine host specificity. Activation of Nod factor receptors triggers growth changes in the root hair to trap a small number of bacteria, which would give rise to the entire population colonizing the resulting nodule.

3. Possibly downstream of Nod factors, rhizobia also use their surface polysaccharides (such as EPS from S. meliloti, depicted) to modulate host range. The plant receptor(s) are unknown, but may resemble animal receptors for surface polysaccharides from bacterial pathogens.

4. In certain rhizobial strains, NodD also induces the expression of TtsI, which codes for a transcriptional regulator that binds to highly conserved promoter elements, called tts boxes, upstream of operons encoding the type III secretion machinery and effectors. Recognition of these effector proteins by R genes present in some ecotypes or varieties of plants limits host range.

Flavonoid – NodD interaction: an early checkpoint of the symbiosis

The legume – rhizobial symbiosis starts with a molecular dialogue between the prospective symbiotic partners. To initiate this process, the legumes secrete a cocktail of phenolic molecules, predominantly flavonoids, which can passively diffuse across the bacterial membrane. Perception of flavonoid signals by the bacterium results in the activation of a suite of bacterial nodulation (nod) genes, which encode the enzymes required for symbiotic development in most legumes (Oldroyd et al., 2011). Transcription of nod genes is mediated by flavonoid-activated bacterial NodD proteins, which are members of the LysR family of transcription regulators (Long, 1996). NodDs bind to conserved DNA motifs, known as nod boxes, found in the promoter regions of nod genes. Although NodDs are directly involved in flavonoid perception, no direct biochemical evidence has been demonstrated for the existence of a flavonoid – NodD complex formed at the nod box. However, it has been shown that flavonoids stimulate an increase in DNA binding of NodD1
to nod gene promoters in Sinorhizobium meliloti (Peck et al., 2006).

NodDs from different rhizobial species respond to different sets of flavonoids; this is also the case for NodD homologues from the same strains (Broughton et al., 2000; Peck et al., 2006). Since different legumes secrete different types of flavonoids, the ability to trigger NodD-dependent induction of nod genes in response to a specific spectrum of flavonoids defines an early checkpoint for the legume – rhizobial symbiosis. For example, point mutations in nodD from Rhizobium leguminosarum bv. trifolii result in extension of host range due to the induction of nod gene expression by flavonoid inducers, which are normally inactive (McIver et al., 1989). Similarly, transfer of nodD1 of the broad-host-range Rhizobium sp. strain NGR234, which responds to a wide range of flavonoid species, to restricted-host-range rhizobia (e.g. S. meliloti) enables the engineered rhizobia to nodulate the non-legume Parasponia (Bender et al., 1988). The role of NodD in regulation of legume-rhizobia recognition was also evidenced by expressing nodD genes from different species of rhizobia in a strain of S. meliloti where all endogenous nodD family members have been mutated (Peck et al., 2006). It was demonstrated that the capability of a profile of flavonoids to induce nod gene expression is dependent on the source of NodD.

Nod factor perception as a determinant of host specificity

With the exception of certain photosynthetic rhizobia that are able to nodulate their legume hosts without producing Nod factors (Giraud et al., 2007), Nod factors are essential for nodulation in most legumes. Nod factors from different rhizobia share the same chitin-like N-acetyl glucosamine oligosaccharide backbone with a fatty acyl chain at the non-reducing end, but differ in their length of the backbone, the size and saturation of the fatty acyl chain, as well as additional modifications at either end, such as glycosylation and sulfation (Long, 1996). Such decorations on the ends of the Nod factor play a crucial role in determining whether the Nod factor can be perceived by a specific host (Lerouge et al., 1990). The core structure of Nod factor is synthesized by the products of nodABC genes. Mutations in these genes completely abolish symbiosis. Additional genes determine the specific decorations on the Nod factor core, and alterations in these genes often change specificity. For example, abolishing the nodE gene in R. leguminosarum bv. trifolii changes the identity of the fatty acyl chain attached to the Nod factor (Spaink et al., 1991), and this change severely affects symbiosis with Trifolium species while enhancing symbiosis with Pismum sativum and Vicia sativa (Djordjevic et al., 1985; Spaink et al., 1989). Conversely, introducing the nodEFGHQP gene cluster from S. meliloti into R. leguminosarum changed the modifications on Nod factor into the S. meliloti type, and the engineered rhizobium became specific to the S. meliloti host Medicago sativa (Debelle et al., 1988; Faucher et al., 1989).

Perception of the Nod-factor signal in legumes is mediated by Nod factor receptors (NFRs). NFRs are plasma membrane-localized serine/threonine receptor kinases that contain LysM motifs in their extracellular domains (e.g. NFR1 and NFR5 in Lotus japonicus; NFP and LYK3 in Medicago truncatula) (Limpens et al., 2003; Madsen et al., 2003; Radutoiu et al., 2003; Arrighi et al., 2006). These LysM domains are found in a variety of peptidoglycan- and chitin-binding proteins, suggesting their probable role in direct binding of Nod factors.

Corresponding to the Nod factor structure, NFRs are a host determinant of symbiosis specificity. This has been demonstrated by genetic and molecular analyses in pea (Pisum sativum), soybean (Glycine max) and L. japonicus. The first example of cultivar – strain interactions was reported in pea where the cultivar ‘Afghanistan’ restricts nodulation by European Rhizobium strains that could nodulate other pea cultivars. The resistance to nodulation in Afghanistan is controlled by a single recessive gene, named sym2. The sym2 allele interacts with a specific gene, nodX, present in strains that are able to nodulate primitive cultivars like Afghanistan (Davis et al., 1988). The nodX gene product acetylates a Nod factor specifically to condition a compatible interaction with the cultivar Afghanistan (Firmin et al., 1993; Geurts et al., 1997). This ‘gene-for-gene’ interaction is consistent with the finding that SYM2 (likely allelic to SYM37) is located in an orthologous region of Lj-NFR1 and Mt-LYK3 (Limpens et al., 2003; Zhukov et al., 2008). In this case, the sym2 allele can only recognize the Nod factors with a NodX-mediated acetylation at their reducing end, but not non-acetylated Nod factors. Another example is the naturally occurring rj1 gene in soybean. The rj1 allele in soybeans prevents nodulation by most strains of Bradyrhizobium. However, some strains that are incompatible with the Rj1/rj1 genotypes have the ability to nodulate the rj1/rj1 plants (Devine et al., 1980). It was recently demonstrated that Rj1 is a soybean orthologue of Lj-NFR1 (Indrasumunar et al., 2011).

The role of NFRs in determining host specificity was also evidenced by the observation that transferring the Lj-NFR1 and Lj-NFR5 to M. truncatula enables nodulation of the transformants by the L. japonicus symbiont Mesorhizobium loti (Radutoiu et al., 2007). Furthermore, the specificity for different rhizobial symbionts of two different Lotus species is a function of a single amino acid residue within one of the LysM domains of Lj-NFR5 (Radutoiu et al., 2007).
Role of rhizobial surface polysaccharides and plant lectins in regulation of host specificity

Another class of bacterial components that can interact directly with the host is bacterial surface polysaccharides, including exopolysaccharides (EPS), lipopolysaccharides (LPS), capsular polysaccharides (KPS) and cyclic β-glucans. As constituents of the bacterial cell wall, they have been reported in numerous studies as symbiotically important. Depending on the particular system, a defect in surface polysaccharides may cause failures of symbiosis at either early or late stages. In the relatively narrow host range bacteria S. meliloti, defects in EPS production tend to result in arrests at the microcolony or infection thread stage (Finan et al., 1985; Leigh et al., 1985), and the severity of the defects correlates with the degree of alteration in the EPS structure (Cheng and Walker, 1998). Similarly, a failure to produce or export cyclic β-glucans leads to the lack of infection threads during symbiosis (Dylan et al., 1986). Several strains of S. meliloti can distinguish between two ecotypes of M. truncatula, producing normal nodules on one ecotype but defective ones on the other. Interestingly, the phenotype can be switched by exchanges of the EPS biosynthesis locus, supporting the notion that surface polysaccharides are specificity determinants (Simsek et al., 2007).

The association of surface polysaccharides with symbiosis specificity at the nitrogen-fixing phase was also demonstrated in other legume – rhizobial interactions. For example, EPS mutants of M. loti produce non-functional nodules on Lotus leucocephala, but are fully effective on L. pedunculatus (Hotter and Scott, 1991). In B. japonicum, an exoB mutant cannot fix nitrogen in Glycine soja, but behaves like wild-type in G. max (Parniske et al., 1994). In R. leguminosarum, some LPS mutants behave normally on pea, while others are defective in nitrogen fixation (Kannenberg et al., 1992).

From the host side, plant lectins have long been speculated as receptors for rhizobial surface polysaccharides and thus are a determinant of host range (Bohlool and Schmidt, 1974). Lectins are a family of carbohydrate-binding proteins with a diverse array of functions, including involvement in host defence against pathogens and symbiosis with soil microbes (De Hoff et al., 2009). Binding of plant lectins to surface polysaccharides has been shown to play a role in promoting rhizobial attachment to root hairs, thereby enhancing the delivery of Nod factors to the root hairs for bacterial infection and nodule initiation (van Rhijn et al., 2001; Laus et al., 2006). The observations that between-species transfer of lectin genes can lead to host range extension support the proposal that plant lectin – surface polysaccharides interactions are associated with host specificity (De Hoff et al., 2009). However, in most cases such host range extension was dependent on similar Nod-factor structures of tested rhizobial strains. Thus, the role that plant lectin plays in root nodule symbiosis remains elusive.

Role of host immunity in determining symbiosis specificity

The plant immune system is comprised of multiple inducible barriers against microbe attack (Jones and Dangl, 2006). In the initial phase, perception of microbe/pathogen-associated molecular patterns (MAMPs/PAMPs) by host pattern recognition receptors (PRRs) triggers PAMP-triggered immunity (PTI). Many Gram-negative pathogens of plants use a type III secretion system (T3SS) to deliver effectors into their hosts to dampen PTI and gain access to the host. In a second layer of defence, plant resistance (R) gene products perceive the presence or action of a corresponding effector protein of the invading microbe to initiate so called effector-triggered immunity (ETI). Therefore, plant defence and microbe-encoded effectors are determinants of host range for pathogens.

Defence responses also occur in the initial phase of the compatible legume – rhizobial interactions, but these responses appear to be less pronounced once the symbiosis was established (Lohar et al., 2006). It was proposed that rhizobial MAMPs, such as Nod factors and surface polysaccharides, could play a role in suppression of defence responses in compatible legume hosts (Shaw and Long, 2003; D’Haeze and Holsters, 2004; Tellström et al., 2007; Jones et al., 2008). In contrast, these rhizobial MAMPs can elicit defence responses in non-legumes (Staehelin et al., 1994), suggesting that rhizobia have evolved specific MAMPs that are recognized only by compatible legume hosts for the purpose of symbiosis development.

Indeed, putative legume Nod-factor receptors have their counterparts in non-legumes (Zhu et al., 2006). These non-legume orthologues, in addition to their potential function in mycorrhizal symbiosis (Op den Camp et al., 2011), play a critical role in chitin signalling and fungal disease resistance (Miya et al., 2007; Wan et al., 2008). Intriguingly, in the interaction between L. japonicus and M. loti, limited alterations in the receptors can lead to the shift of the intracellular signalling from defence to symbiosis (Nakagawa et al., 2011). These studies suggested that defence and symbiotic pathways overlap in legumes, which was also evidenced by comparing the responses of L. japonicus to M. loti and to the conserved bacterial effector flg22 (Lopez-Gomez et al., 2011). Thus, to establish a symbiotic association, rhizobia need to evade or suppress the defence response of a legume species and turn it to a host. Non-host resistance associated with non-self recog-
nition of rhizobial MAMPs may explain host specificity across species.

A significant similarity between pathogenic and symbiotic bacteria is that many, but not all, rhizobial strains also use the type III secretion system (T3SS) to deliver effectors, so called nodulation outer proteins or Nops, into the host cells (reviewed in Deakin and Broughton, 2009). Similar to the synthesis of Nod factors, the synthesis of rhizobial T3SS and its secreted effectors is also regulated by flavonoids and the bacterial transcription activator NodD. NodD, in this case, induces the expression of tssl, a gene encoding a transcriptional regulator that binds to highly conserved promoter elements, called tts boxes, upstream of operons encoding the secretion machinery and effectors (Wassem et al., 2008). The rhizobial type III effectors have been shown to play an important role in the modulation of host range. In contrast to T3SSs of bacterial pathogens, which are required for both pathogenicity and eliciting defence response (Buttner and He, 2009), rhizobial T3SSs are dispensable for rhizobial infection and nodulation. By analogy with plant – pathogen interactions, it has long been speculated that rhizobial type III effectors may also be perceived by host R genes to trigger ETI that regulates cultivar-specific nodulation (Sadowsky et al., 1990; Deakin and Broughton, 2009; Soto et al., 2009).

Dominant genes that restrict nodulation with specific rhizobial strains have been identified in soybeans and other legumes (reviewed in Devine and Kuykendall, 1996; Parker, 1999). The dominant nature of these genes is in contrast to those 'loss-of-recognition' recessive alleles in Nod-factor receptors (Geurts et al., 1997; Zhukov et al., 2008), but resembles gene-for-gene resistance against plant pathogens. Using map-based cloning, the Rj2 and Rfg1 genes have recently been cloned in soybeans (Yang et al., 2010). The Rj2 gene restricts nodulation with specific Bradyrhizobium japonicum strains such as USDA122, whereas Rfg1 prevents nodulation with certain fast-growing Sinorhizobium fredii strains such as USDA257. It turns out that Rj2 and Rfg1 are allelic genes encoding a member of the Toll-interleukin receptor/nucleotide-binding site/leucine-rich repeat (TIR-NBS-LRR) class of plant resistance proteins.

A survey of various soybean genotypes identified three types of alleles based on their responses to B. japonicum USDA122 and S. fredii USDA257: the Rj2 (rgf1) allelic type that allows for nodulation with USDA257 but not with USDA122; the rj2 (Rfg1) allelic type that permits nodulation with USDA122 but not with USDA257; and the rj2 (rgf1) allelic type that enables nodulation with both USDA122 and USDA257. The Rj2 (Rfg1) allelic type defined as resistance to nodulation with both strains was not identified. These results suggest that soybean plants carrying an Rj2 or Rfg1 allele commonly show contrasting specificity with USDA122 and USDA257 and thus can form symbiotic interactions with at least one of the two strains. Sequence analysis of these alleles revealed polymorphisms in the NBS and LRR domains that correlated with the restriction of B. japonicum or S. fredii.

The involvement of R genes in the control of nodulation specificity is in line with the observation that many rhizobial strains possess T3SSs to deliver effectors into the host cells (Deakin and Broughton, 2009). Some rhizobial effectors are homologous to those secreted by bacterial pathogens, suggesting that symbiotic and pathogenic bacteria share a similar strategy to invade the host (Dai et al., 2008; Kambara et al., 2009). Certain rhizobial effectors may promote bacterial infection when not recognized by the host R genes, but function negatively if perceived by the host immune system. In the incompatible interactions controlled by the Rj2 or Rfg1 alleles, early responses such as root hair curling can be observed but the infection process was completely blocked, which was presumably caused by defence responses triggered by the recognition of yet unknown rhizobial effectors. This hypothesis is supported by the fact that a TSS mutant of S. fredii USDA257 can restore nodulation with the soybean genotypes carrying the Rfg1 alleles.

The dominant nodulation-restrictive R genes are widespread in natural populations of legumes (Parker, 1999). This raises the question of why the host maintains a gene that restricts a beneficial interaction. First, this may occur as a result of balancing selection, because these types of R genes may guard a common virulence target of pathogens and symbions, providing the host a dilemma between forming symbiosis and mounting defence responses. Eventually natural selection would maintain both allelic types to balance benefits and costs, depending on environmental conditions in which the plants are grown. Alternately, some host genotypes may have evolved R genes to deterministically interact with certain strains with high nitrogen fixation efficiency but exclude those with low nitrogen fixation efficiency.

Natural variation in strain-specific nitrogen fixation: an important but less explored topic in root nodule symbiosis

Although the legume – rhizobial interactions are overall beneficial to the host, the beneficial function (i.e. nitrogen fixation efficiency) varies tremendously with different plant – rhizobial combinations (Schumpp and Deakin, 2010). In an extreme situation, certain rhizobial strains can nodulate a host genotype but are unable to fix nitrogen (Nod + Fix –); however, the same strains can establish an efficient interaction (Nod + Fix +) with alternative host genotypes (Tirichine et al., 2000; Simsek et al., 2007). Despite recent advances in our understanding of the signalling pathways leading to initial recognition and
nodule development (Madsen et al., 2010; Oldroyd et al., 2011), the molecular mechanisms underlying strain-specific nitrogen fixation are largely unknown.

Specificity in nitrogen fixation has been well documented in the *M. truncatula* – *S. meliloti* interaction (Snyman and Strijdom, 1980; Tirichine et al., 2000; Parra-Colmenares and Kahn, 2005; Simsek et al., 2007; Rangin et al., 2008). Genetic analysis has identified a single gene (*Mt-Sym6*) that conditions host-specific nitrogen fixation upon inoculation with the *S. meliloti* strain A145 (Tirichine et al., 2000). In *R. leguminosarum* bv. *trifolii*, strains have different compatibility with white clover and Caucasian clover. Strain ICC105 produces non-fixing nodules on the former, while nodulating effectively on the latter. Genetic studies demonstrate that in incompatible interactions, promoter sequences unique to ICC105 prevent the NifA protein from activating genes required to assemble the nitrogenase enzyme (Miller et al., 2007). How the activity of NifA is regulated is unknown.

Despite a lack of knowledge about the gene networks that govern host specificity in the nitrogen-fixing process, several scenarios could be envisioned. First, beyond the initial molecular dialogue leading to bacterial entry and nodule formation, the symbiotic partners likely undergo additional rounds of molecular communication within mature nodules. This later signal exchange could contribute positively or negatively (e.g. triggering defence-like responses) to the differentiation and/or persistence of bacteroids and symbiosomes, and accordingly affect nitrogen fixation efficiency. Second, there may exist host genes that regulate host-specific expression of bacterial genes associated with nitrogen fixation. Additionally, since bacteroids and symbiosomes have complete metabolic dependence on their hosts, the status of metabolic changes (e.g. amino acid cycling) across the peribacteroid membrane may also play a critical role in regulating the effectiveness of nitrogen fixation. Research is needed to elucidate the complexity of this important, but currently overlooked, aspect of the legume – rhizobial symbiosis, because of its direct relevance to sustainable agriculture. Such knowledge will help to develop strategies to enhance the agronomic potential of biological nitrogen fixation.

**Concluding remarks and future perspectives**

Multiple checkpoints are employed during the course of the legume – rhizobial symbiosis. Such interactions depend on exported Nod factors, surface polysaccharides and secreted proteins from the bacteria. Often the presence of these factors is essential for symbiosis, and changes in their structure or sequence alter host specificity. It is clear that where the interaction is best characterized, we also know the most about how specificity is achieved. In the case of Nod factor – NFR interaction, specificity can be defined by specific modifications and individual amino acids. A large number of bacterial secreted proteins have been catalogued (Deakin and Broughton, 2009), and we are at the beginning of defining the host factors recognizing these bacterial effectors (Yang et al., 2010). However, the nature of the interaction between these two components is still unknown. If the parallel with host defence response holds true, the host targets of symbiotic bacterial effectors remain to be identified. With bacterial surface polysaccharides, the effects of modifying or abolishing certain components are well demonstrated, while the host receptors to these molecules (or other specificity determinants from the host) remain elusive. Therefore, identifying genes contributing to symbiotic specificity, primarily from the host, should contribute substantially to our knowledge of this phenomenon.

Advances in understanding specificity in symbiosis will likely be facilitated by the shift from inter-species studies to characterizing ecotype differences within the same species. Ecotypes showing differential responses to the same collection of rhizobia can be crossed to follow the loci of major effects. It is possible that the factors determining specificity across species are not fundamentally different from those within species. T3SSs and their secreted effector proteins not only determine the outcome between *Rhizobium* sp. NGR234 and various legume species, but also dictate the cultivars of soybean that *S. fredii* USDA257 can nodulate (Viprey et al., 1998; Yang et al., 2010). Similarly, changes in EPS alter the ability of *M. loti* to interact with different *Lotus* species, but are also implicated in ecotype-specific interaction between *S. meliloti* and *M. truncatula*. Identifying host determinants of ecotype specificity would provide candidate genes for inter-species specificity, which can be investigated much in the same manner as structure-function studies in nod factor receptors (Radutoiu et al., 2007).

Studying symbiosis specificity within species morphs into the realm of natural variation. Such specificity has been well documented in soybean, but is relatively under-investigated in model legumes. If studies in Arabidopsis can serve as examples, an exercise through natural variation will not only provide new insights into known symbiosis genes, but also lead to the identification of new players somehow missed by more traditional approaches. Given their technical advantages, exploring the full potential of model systems should lead to more rapid cloning of host specificity genes. Of particular interest is the Medicago HapMap project (http://medicagohapmap.org), where the genomes of several hundred *M. truncatula* lines are being sequenced. Such a torrent of sequence information, combined with a grid detailing the symbiotic phenotype of each line with a collection of rhizobial strains, will promise...
accelerated identification of single specificity genes or major quantitative trait loci through association mapping.

One tantalizing observation is the polymorphism at genomic loci encoding the nodule-specific cysteine-rich (NCR) peptides (Van de Velde et al., 2010). NCRs are defensin-type antimicrobial peptides that are targeted to the bacteria and act as symbiotic plant effectors to direct the bacteroids into a terminally differentiated state. Each Medicago genome encodes hundreds of NCR peptides, with divergent primary sequences. One hypothesis explaining the large number of NCRs is that they are needed to interact with the myriad rhizobial species that a host would encounter in the wild. The realization that the NCR loci are comparably polymorphic with R genes involved in the recognition of diverse pathogenic microbes is yet another interesting parallel between symbiosis and defence. Could the NCRs be specificity determinants at the nitrogen fixation stage of root nodule symbiosis?

Because the legume–rhizobial symbiosis involves two partners, specificity determinants should be pursued in a two-pronged approach. Given the expanding tool kits available to each organism, one can be confident that advances in one organism will spur breakthroughs in the other, synergistically leading to a comprehensive understanding of the molecular mechanisms underlying specificity in symbiosis.

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