Published in final edited form as:
Annu Rev Genet. 2008 ; 42: 413–441. doi:10.1146/annurev.genet.42.110807.091427.

Molecular Determinants of a Symbiotic Chronic Infection

Katherine E. Gibson[1,2], **Hajime Kobayashi**[2], and **Graham C. Walker**[2]
Katherine E. Gibson: ; Hajime Kobayashi: ; Graham C. Walker: gwalker@mit.edu
[2] Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Abstract

Rhizobial bacteria colonize legume roots for the purpose of biological nitrogen fixation. A complex series of events, coordinated by host and bacterial signal molecules, underlie the development of this symbiotic interaction. Rhizobia elicit de novo formation of a novel root organ within which they establish a chronic intracellular infection. Legumes permit rhizobia to invade these root tissues while exerting control over the infection process. Once rhizobia gain intracellular access to their host, legumes also strongly influence the process of bacterial differentiation that is required for nitrogen fixation. Even so, symbiotic rhizobia play an active role in promoting their goal of host invasion and chronic persistence by producing a variety of signal molecules that elicit changes in host gene expression. In particular, rhizobia appear to advocate for their access to the host by producing a variety of signal molecules capable of suppressing a general pathogen defense response.

Keywords

Rhizobium; legume; bacterial invasion; signaling; stress response; cell cycle

INTRODUCTION

Although nitrogen is one of the most abundant elements present on Earth, it is also one of the most limiting for biological growth because it is largely found in the inaccessible form dinitrogen (N_2). Biological nitrogen fixation is the process by which chemically inert N_2 present in the atmosphere is enzymatically reduced to the metabolically usable form ammonia (NH_3) through the action of nitrogenase (129). The ability to catalyze the conversion of N_2 to NH_3 has evolved only among microbes, including the rhizobia, cyanobacteria, azobacteria, frankia, and archaea (137).

This review focuses on developmental events that underlie biological nitrogen fixation within the context of an intimate symbiosis between rhizobia, a phylogenetically diverse group of gram-negative soil bacteria within the family *Rhizobiaceae*, and their hosts the *Leguminosae* (or *Fabaceae*) family of flowering plants. This rhizobium-legume symbiosis is established under nitrogen-limiting soil conditions and is estimated to contribute nearly half of all current biological nitrogen fixation (68). In addition to its environmental and agricultural significance (170), this symbiosis provides a tractable model system for identifying and characterizing certain mechanisms employed by invasive bacteria during chronic host interactions as they transition from a free-living environment to their niche within the host. In fact, many rhizobial

Correspondence to: Graham C. Walker, gwalker@mit.edu.
[1]Present address: Department of Biology, University of Massachusetts, Boston, Massachusetts 02125

DISCLOSURE STATEMENT
The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

genes required for either host invasion or chronic persistence have orthologs in closely related alphaproteobacterial pathogens, such as *Agrobacterium* and *Brucella*, and often these orthologs have an effect on virulence (11,140,156). Moreover, plants can detect phytopathogens using receptors that are evolutionarily related to those employed by legumes to detect symbiotic rhizobia (144,180). Thus, the broad importance of this symbiosis is ever growing.

Due to the wide breadth of biological processes involved in establishing the legume-rhizobium symbiosis, as well as the natural diversity in symbiotic mechanisms, we are unable to review all the events that underlie this complex interaction. For instance, not discussed here are the Type III and Type IV secretion systems that play an important role in diverse rhizobial symbioses (32,112), nor the bacterial quorum sensing systems that modulate symbiotic interactions (67,145). In addition, it is clear that plant hormone balance plays a critical role in regulating nodule development (122). Rather, our goal is to provide the reader with molecular insight into several regulatory aspects of this symbiosis while providing reference to reviews that discuss these processes in more detail.

EVOLUTION OF SYMBIOSIS

The rhizobium-legume symbiosis, a relatively recent evolutionary adaptation, is thought to have evolved from the ancient arbuscular mycorrhizal symbiosis that is nearly ubiquitous throughout the plant kingdom and provides plants with the essential mineral nutrient phosphorus (73). This evolutionary relationship has been inferred based on findings that several host genes represent common requirements for the establishment of both rhizobial and mycorrhizal symbioses. Given that nearly all vascular plants interact with mycorrhizal symbionts, it remains unclear why the nitrogen-fixing symbiosis is strictly limited to legume species, with the exception of *Parasponia*. Current understandings of legume evolution and the appearance of nodulation indicate that the first symbiosis event involved bacterial invasion of roots via cracks in the host epidermis where lateral roots emerge (158). Subsequent to this, developmental mechanisms evolved, likely through the process of gene duplication, to craft the highly selective symbiosis described here. In particular, the emergence of a host-derived infection structure allows host control over the bacterial infection process (59).

The symbiotic capacity of rhizobia is thought to have evolved in part through horizontal gene transfer events based on several observations. Within the symbiotic rhizobial lineage, *Sinorhizobium* is estimated to have diverged from *Bradyrhizobium* approximately 500 Mya (168), which is well before the initial appearance of legume species approximately 60 Mya (158). Rhizobia also tend to have large and multipartite genomes consisting of a chromosome supplemented with one or more independent plasmids (81), which contribute to an evolutionarily dynamic genome through the process of horizontal gene transfer. Moreover, rhizobial genes involved in symbiosis are often located within chromosomal islands or on plasmids: *Sinorhizobium meliloti* genes involved in NF biosynthesis (*nod*, *nol*, *noe*) and nitrogen fixation (*nif* and *fix*) are located on pSymA whereas others required for EPS (exopolysaccharide) biosynthesis (*exo*) and C_4-dicarboxylic acid utilization (*dct*) are located on pSymB (60). Horizontal transfer of these genomic elements has been observed among bacteria within the rhizosphere and has the ability to convert a nonsymbiont into a symbiont through a single transfer event (6,160,161). Other than symbiosis genes, there is no significant synteny shared between the plasmids of various rhizobial species or rhizobial chromosomes (19,188). Finally, it was recently discovered that certain betaproteobacteria are also capable of establishing nitrogen-fixing symbioses with legumes (117), and comparative phylogenetic analyses support the notion that the plasmid-borne symbiotic genes (*nod* and *nif*) in these *Burkholderia* are derived from at least one horizontal gene transfer event (25).

NODULE DEVELOPMENT

To establish the symbiosis, free-living soil rhizobia elicit de novo formation of a specialized root organ, the nodule, from their host. The root nodule ultimately houses many thousands of individual rhizobia that catalyze nitrogen fixation. Organogenesis of the nodule occurs via a developmental program that involves dedifferentiation of quiescent (G_0) root cortical cells into an actively dividing meristem (Figure 1*a*), which is functionally analogous to an animal stem cell population (159). These dividing meristem cells form the initial nodule primordium from which a mature nodule develops (Figure 1*a*). A subset of these meristem cells enter a modified cell cycle program that results in endoreduplication and produces greatly enlarged cells. It is this subset of endoploid cells that are invaded and colonized by rhizobia for the purpose of nitrogen fixation (Figure 1*d*).

To colonize the developing nodule, bacteria present in the soil provoke root hair curling by the host (Figure 1*a*,*b*). The root hair curl traps intimately associated bacteria, which are then able to invade the nodule through a host-derived structure called the infection thread (IT) (Figure 1*a*,*c*). The IT is a tube originating at the tip of the root hair that initially forms via localized hydrolysis of the cell wall and that continues its growth via deposition of new cell wall material (59). In this way, the IT carries bacteria from the root surface toward the population of newly created endoploid cells where it undergoes significant ramification. Ultimately, bacteria within the IT are deposited into the host cell cytoplasm in a process that resembles endocytosis; they then enter a differentiation program that results in their ability to catalyze nitrogen fixation.

Generally, nodules fall into two morphological classes based on their pattern of meristem growth: indeterminate, with a meristem derived from inner cortical cells, and determinate, with a meristem derived from outer cortical cells. Indeterminate and determinate nodules also differ in the relative persistence of meristem proliferation. In determinant legumes, including most Old World tropical species and *Lotus japonicus*, the basal meristem undergoes a limited spurt of cell division such that further nodule growth depends on an expansion in cell size rather than in cell number. Both plant and bacterial development proceeds synchronously within this particular type of nodule; an exception is senescence, which occurs within older nodules on an individual cellular basis (133). Thus, a determinate nodule contains a relatively homogenous population of developing plant and bacterial cells at any given time point, which allows precise analysis of temporal gene expression and function within both symbiotic partners (Figure 1*e, f*). In contrast, legumes that form indeterminate nodules, including most temperate species such as *Medicago truncatula*, create a persistent meristem. In these nodules, the plant allows prolonged bacterial invasion of postmitotic (G_0) endoploid cells that are continuously generated by the meristem. As a result, each developmental stage required to establish the symbiosis is present within a single mature indeterminate nodule and in a spatially organized manner (Figure 1*a*). The distinct regions found in a mature indeterminate nodule, proximal to distal from the root, are the meristem (Zone I), the invasion zone (Zone II), the interzone (Zone II/III), the nitrogen-fixing zone (Zone III), and the senescent zone (Zone IV) (172). In addition to distinct morphological features, these zones can be distinguished by patterns of host gene expression. While much research has focused on plant and bacterial physiology in Zones I–III, comparatively less is known regarding the genetic regulation and physiology of senescence in Zone IV although this process is also of critical agricultural consequence (133).

BACTERIAL INVASION

There is often strict specificity in the establishment of a nitrogen-fixing symbiosis between host legume species and their symbionts. For instance, the bacterium *S. meliloti* is compatible with species of *Medicago* (alfalfa), *Melilotus* (sweetclover), and *Trigonella* (fenugreek), whereas *Rhizobium etli* is compatible only with species of *Phaseolus* (bean). In contrast to

these restricted host range rhizobia, some bacteria have a broad host range, like *Rhizobium* sp. NGR234, which is capable of nodulating 232 legumes from 112 distantly related genera (132). Based on rhizobial phylogenetic relationships, it was suggested that the restricted host range symbiosis evolved from an ancestral broad host range symbiosis (132), and perhaps the specificity engendered by narrow host range interactions creates a finely tuned and more effective symbiosis.

The Host Flavonoid Signal

Compatible rhizobia are uniquely capable of gaining entry and invading the host nodule based on a series of reciprocal signaling events (Figure 1*b*). The principal signals originating from the host and perceived by rhizobia in the soil are derived from the flavonoid family of secondary plant metabolites (Figure 2*a*). The roots of leguminous plants exude a diverse cocktail of flavonoids and isoflavonoids into the soil (128). Compatible bacteria in the rhizosphere can elicit quantitative increases and qualitative changes in flavonoid exudation. Exactly which flavonoid in the rhizosphere a compatible bacterium perceives can be difficult to determine since plants secrete a complex mixture. In fact, it is quite likely that the spectrum of flavonoids exuded by a legume, rather than a single flavonoid compound, provides a determinant for host specificity. At least some rhizobia are chemotactic toward compatible flavonoids, suggesting that certain aspects of host specificity are established before the bacterium and its host physically interact (20).

Host Flavonoids Are Perceived by Bacterial NodDs

Plant-derived flavonoids elicit a significant transcriptional response from compatible bacteria within the rhizosphere, most of which is NodD-dependent and results in Nodulation Factor (NF) signal production (Figure 2*b*) (23). While expression of genes required for NF synthesis is induced by host flavonoids, expression of these same genes can be repressed in the presence of ammonia.

NodD belongs to the LysR family of DNA-binding transcription factors, which have an N-terminal ligand-binding domain that regulates the activity of the associated C-terminal DNA-binding domain. The NodD ligand-binding domain is thought to function as a flavonoid receptor and, in the presence of a compatible host, NodD induces the expression of genes involved in NF biosynthesis. For example, the daidzein and genistein isoflavonoids of *Glycine max* (soybean) induce NF gene expression in *Bradyrhizobium japonicum* (Figure 2*a*). However, daidzein prevents NF production in the noncompatible bacterium *S. meliloti*, which responds positively to the flavone luteolin (Figure 2*a*), and does so in a NodD-dependent manner (127). Generally, the NodDs of broad host range rhizobia respond to a wider range of flavonoid species than those present in restricted host range bacteria (128). For instance, NodD1 from the broad host range symbiont *Rhizobium* sp. NGR234 responds positively to a structurally diverse range of compounds, including phenolics (vanillin and isovanillin) that are inhibitors for other rhizobia (97). The transfer of *Rhizobium* sp. NGR234 *nodD1* to a restricted host range rhizobium, like *S. meliloti*, extends the spectrum of plants the transconjugant recognizes as a symbiotic partner (16).

NodD regulates transcription by binding *cis*-acting regulatory sequences called *nod*-boxes and these elements are generally found upstream of the promoters of the *nod*, *nol*, and *noe* genes involved in NF production (8,82). However, interesting nuances to NodD-dependent regulation are beginning to emerge, including the identification of genes unrelated to NF biosynthesis within the NodD regulon (87,109,165), and a temporal progression to flavonoid-induced gene expression that implies NodD coordinates a complex regulatory hierarchy (87).

The Bacterial NF Response

Bacterial NF functions as a key that opens the door to its host (128), meaning there is a high degree of stringency for NF chemical structure that determines whether the host allows bacterial invasion to proceed. NF also functions as a mitogen and modifies the plant hormone balance to elicit the primordium formation that ultimately gives rise to mature nodule tissue (Figure 1*a*) (37,56,122). It was recently shown that NF also plays a role in *S. meliloti* biofilm development and in a host-independent manner (58); thus, NF appears to perform a significant role in both free-living and symbiotic lifestyles.

NF is a complex signaling molecule secreted from the cell as a cocktail of β-(1,4)-linked N-acetyl-D-glucosamine (GlcNAc) trimers, tetramers, or pentamers (Figure 2*b*) (37). The chitin backbone is modified on the nonreducing terminal residue at the C2 position by a fatty acid; however, the size and saturation-state of this lipid chain varies in a species-specific manner. NF can be further decorated with a variety of chemical substituents, including acetyl, arabinosyl, carbamoyl, fucosyl, methyl, and sulfuryl additions. In fact, a given rhizobial species will produce a mixture of NF compounds, anywhere from 2 to 60 distinct molecules, and this is especially true of broad host range bacteria (128).

The diversity of NF structures produced by rhizobia derives from the combined presence of species-specific genes and allelic variation of the common nodulation genes (128). Common *nod* genes (including *nodA, B*, and *C*), which are found in nearly all rhizobial species and are capable of cross-species complementation, are responsible for synthesis of the NF chitin backbone. In contrast, host-specific *nod* genes confer specificity for nodulation of a particular host and are involved in various modifications of the chitin backbone. The ability to synthesize and secrete NF can be transferred to *Escherichia coli* by introduction of the NF biosynthetic gene cluster, and this confers upon *E. coli* the ability to elicit several of the early NF host responses described below (178).

Host Responses to NF

A number of physiological responses to NF are observed when either bacteria or purified NF are applied to roots (37), and these responses have been used to position host genes within a signaling pathway (122). Purified NF is effective at eliciting most host responses at nanomolar concentrations. Initial root epidermal responses include an alkalinization of the cytosol, and a depolarization of the plasma membrane within minutes of root inoculation. These two responses appear to depend on a brief NF-induced Ca^{2+}-influx that precedes them by seconds, and they are closely followed by a prolonged Ca^{2+}-spiking response that lasts between 20 to 60 min. Purified NF is also sufficient to induce root hair deformation and root hair curling within a few hours of application. Root hair deformation likely relies on Ca^{2+}-induced changes to the organization of the actin cytoskeleton, which produce a reorientation of cell growth. In fact, NF can accumulate within the host plasma membrane (66), and appears to provide a direct positional cue to the host such that the tip of the root hair grows toward the site of greatest NF concentration (51).

NF elicits significant changes in the expression of host genes, including those induced early in nodule development that are referred to as early nodulin, or *ENOD*, genes (37,122). More globally, transcriptome profiles reveal that plant genes predicted to be involved in responses to abiotic and biotic stresses, as well as cell reorganization and proliferation, are rapidly induced by rhizobia and largely in a NF-dependent manner (49,105,116). At the earliest postinoculation time point of 1 h, *M. truncatula* genes that encode functions related to abiotic stress and disease resistance are upregulated while those involved in translation and cytoskeletal organization are downregulated (105). Abiotic stress and disease resistance genes become significantly downregulated by 6 h postinoculation and this trend continues until at least 3 days

postinoculation (105). As discussed below, some of this regulation may be enhanced by bacterial EPSs, cyclic β glucans and LPSs. Concurrent with a dramatic reorientation of root cell growth, genes involved in cytoskeletal functions and cell-wall biogenesis are induced from 1–12 h postinoculation (105). Between 12–48 h, genes involved in translation, cell growth and division, and chromosomal organization are upregulated in a manner consistent with the initiation of meristem proliferation within the root cortex (105,116). As one might predict, increased expression of cell division genes is transient within a determinate nodule and decreases concurrent with the onset of nitrogen fixation (29,90).

The importance of NF structure for biological activity has been demonstrated through biochemical analyses of NFs produced by host-specific *nod* gene mutants and the corresponding phenotypic characterization of plant responses. For instance, the first symbiotically active NF structure described was that produced by *S. meliloti*, which is a chitin tetramer with an N-linked C16 unsaturated fatty acid and both O-sulfuryl and O-acetyl modifications (Figure 2*b*) (149). An *S. meliloti* mutant deficient for the host-specific *nodH* gene lacks the O-sulfuryl substitution at the reducing terminus of its NF and correspondingly loses the capacity to nodulate its natural *M. truncatula* host, yet it acquires the novel ability to nodulate *Vicia hirsute* (139). The purified nonsulfated NF produced by a *nodH* mutant elicits Ca^{2+}-spiking from its natural host only when present at concentrations much greater than is required for wild-type NF (123, 139, 178), suggesting the sulfate modification contributes to host range specificity by modulating the affinity between NF and its host receptor.

While the *S. meliloti nodH* gene is required to elicit initial physiological responses from *M. truncatula*, the host-specific *nodEF* (NF acylation) and *nodL* (NF acetylation) genes are subsequently involved in host invasion. Individual null mutants have a delayed nodulation phenotype associated with inefficient bacterial invasion, whereas the *nodFL* double mutant has a more severe defect in infection thread formation (5,103). Despite the block in bacterial invasion, a *nodFL* double mutant (and its purified NFs) triggers certain morphological responses from root hairs in a manner indistinguishable from the wild type. These include the early Ca^{2+}-spiking and root hair curling responses, as well as induction of certain *ENOD*s and nodule primordium formation (5,24,178). Thus, in *M. truncatula* there are distinct structural requirements for early NF-dependent root hair responses (i.e., NF sulfation) vs later NF-dependent infection events (i.e., NF sulfation, and acetylation or acylation).

The relative ease with which plant symbiosis mutants can now be characterized molecularly has significantly expanded our understanding of the host signal transduction pathway that allows bacterial invasion based on NF perception (78,122). Putative NF-receptors, including MtNFP and MtLYK3, belong to the lysin motif (LysM) receptor-like kinase family, which have an extracellular domain homologous to the bacterial LysM proteins that bind β-(1,4)-linked GlcNAc derived from peptidoglycan. Host responses to specific NF structures depend on the LysM domain specifically, and a one amino acid difference within this motif can alter the range of rhizobia recognized for symbiosis (134).

Additional NF signaling genes have been identified that function downstream of the LysM receptor-like kinases (78,122). For example, several *dmi* (does not make infections) mutants have been characterized in *M. truncatula*, and the affected genes have orthologs in *L. japonicus*. Highlighting the importance of the NF-dependent Ca^{2+}-spiking response, *MtDMI3* encodes a putative Ca^{2+}-calmodulin-dependent protein kinase (CCaMK) (115). *MtDMI1* encodes a nuclear-localized cation channel that may help modulate Ca^{2+}-spiking, and *MtDMI2* encodes a putative receptor-like kinase (76,119). While all of the *dmi* mutants display root hair deformation and rapid Ca^{2+}-influx in response to NF, *dmi1* and *dmi2* mutants are unable to elicit the subsequent Ca^{2+}-spiking response (50,72,75,159,177,179). In contrast, the *dmi3* mutant is indistinguishable from wild type with regard to each of these physiological NF

responses (177). Thus, *M. truncatula DMI1* and *DMI2* appear to function downstream of *MtNFP* and *MtLYK3*, but upstream of the Ca^{2+}-calmodulin-dependent protein kinase encoded by *DMI3*. Recent reports showing that *DMI3* can be genetically modified to elicit spontaneous nodule formation suggest it may be possible to intelligently engineer non-legume species that are capable of establishing symbiotic nitrogen fixation (65,166).

Although NF signaling is a nearly universal means of establishing the rhizobium nitrogen-fixing symbiosis with compatible legumes, exceptions are emerging. The recent sequencing of photosynthetic *Bradyrhizobium* sp. BTAi1 and ORS278, which form nitrogen-fixing nodules on the roots and stems of aquatic host, *Aeschynomene sensitiva*, revealed that the common *nodABC* genes are absent in these species (63). Moreover, a NF-deficient mutant of the closely related *Bradyrhizobium* sp. ORS285 forms nitrogen-fixing root and stem nodules on *A. sensitiva* with the same efficiency as its wild-type parental strain. Thus, the host *A. sensitiva* initiates nodule development in a NF-independent manner and instead may respond to the secretion of bacterial purine derivatives with cytokinin-like activity, highlighting the importance that host hormone balance plays in nodule formation (63).

REACTIVE OXYGEN AND NITROGEN SPECIES

As with many host-microbe interactions (39,118,187), the rhizobium-legume symbiosis can be associated with a host-generated release of reactive oxygen species (ROS: $O_2^{\cdot-}$, H_2O_2, and $HO^{\cdot}$) and reactive nitrogen species (RNS: $NO^{\cdot}$). As discussed below, application of purified *S. meliloti* LPS can suppress ROS production in response to fungal elicitors in *M. truncatula* tissue culture cells (1,148); however, the role this response plays in symbiosis is unclear. The application of purified *S. meliloti* NF to *M. truncatula* roots also inhibits ROS production in response to fungal elicitors (151), and correspondingly limits the expression of putative ROS-generating NADPH oxidase genes (104). The NF-mediated inhibition of ROS efflux occurs within 1h of treatment and is dependent on the putative NF receptor encoded by *MtNFP* but not the downstream *DMI1* gene (104,151). NF-mediated suppression of ROS production plays a causative role in the root hair deformation and curling responses to rhizobia (104).

However, subsequent stages of symbiosis between *M. truncatula* and *S. meliloti* result in the long-term production of ROS and RNS in distinct areas of the nodule. While RNS are primarily associated with plant cells that have been infected with bacteria (12), ROS are associated with the plant cell wall of both infection threads and infected host cells (142,147). This ROS efflux requires NF, arguing that ROS may play a positive role in bacterial invasion (136). The *M. truncatula* response to NF includes induction of the *rip1* gene encoding a putative peroxidase that could be involved in hydrogen peroxide-dependent cross-linking of cell wall proteins (31). Both the induction of *rip1* expression and the production of ROS require that *M. truncatula* has a functional *DMI1* gene (136), genetically separating the early *DMI1*-independent decrease in ROS from this later response. The *DMI1* requirement for *rip1* induction is bypassed when root tissues are exposed to exogenous H_2O_2 (136).

Generally, free-living rhizobia are more susceptible to ROS-mediated killing than are other common soil bacteria like *Bacilli* and *Pseudomonads* (120), suggesting that symbiotic levels of ROS are probably unable to differentially prevent soil pathogens from taking advantage of ITs to invade nodules. One role for ROS production may be to promote proper IT development and growth by either cross-linking cell wall glycoproteins or degrading cell wall–associated polysaccharides to aid IT elongation. Only a small percentage of newly formed ITs actually penetrate the inner cortical cell layer, and the unsuccessful, or aborted, ITs display certain characteristics of the hypersensitive plant defense response (171), which typically includes ROS production. Thus, this ROS efflux could play a role in limiting bacterial invasion.

Recent observations are consistent with the idea that ROS may also function as a positive signal perceived by bacteria during invasion. This is based on the finding that an *S. meliloti* strain overexpressing the KatB catalase has a nodule invasion defect that is primarily associated with aberrant IT growth (76). KatB is responsible for detoxifying H_2O_2 and thereby limits the concentration of exogenously applied ROS within the bacterial cell (4). Bacterial overexpression of KatB likely acts as a strong catalyst for detoxifying ROS and may lower the local concentration of free oxygen radicals able to modify the IT compartment. KatB overexpression presumably also leads to significantly decreased concentrations of ROS within the IT-localized bacterial cell. The symbiosis defect associated with KatB overexpression could therefore reflect the fact that ROS functions as a cytoplasmic signal that the bacterium uses to regulate functions essential to invasion. It is unlikely this ROS signal would be perceived through the OxyR redox-sensitive regulatory protein since an *S. meliloti oxyR* mutant has no obvious symbiosis defect with *M. truncatula* (41,107). However, the putative redox-sensitive CbrA two-component histidine kinase is required for bacterial invasion of *Medicago* hosts and regulates a number of genes specifically required for bacterial invasion (61,62), making CbrA a promising candidate for a bacterial redox-sensor involved in bacterial invasion.

Although ROS appears to promote rhizobial invasion, these bacteria must also be able to combat this stress in order to achieve symbiosis. A screen for ROS-sensitive *S. meliloti* mutants that simultaneously display aberrant symbiosis phenotypes revealed a variety of functions related to bacterial metabolism and exopolysac production (41). With regard to genes specifically required to detoxify ROS, *S. meliloti* has three that encode catalase enzymes (*katA*, *katB*, and *katC*) and one encoding superoxide dismutase activity (*sodB*) (77,146). Several uncharacterized genes include an extracellular peroxidase (*Smc01944*), a bacteriocuprein-family superoxide dismutase (*sodC*), and an alkylhydroperoxidase (*ahpC*), each of which may combat ROS exposure; however, null phenotypes for these genes have not been reported (7,60). Null mutants for either *katA*, *katB*, *katC*, or *sodA* are fully capable of establishing the symbiosis (40,77,152), although the *katAC* and *katBC* double mutants have decreased symbiotic proficiency (77,152). In particular, the *katBC* mutant has a severe symbiosis defect that results in formation of aberrantly small nodules that are incapable of nitrogen fixation (77). Although the *katB* and *katC* genes are strongly expressed in bacteria located within growing ITs, where ROS is concentrated, the phenotype of the double mutant is subsequently revealed during bacterial uptake into the host cell cytoplasm: intracellular bacteria lack the surrounding peribacteroid membrane and undergo rapid senescence for reasons that are unclear (77).

MODULATION OF THE HOST DEFENSE RESPONSE

The initiation of ITs is a major checkpoint for the host in terms of deciding whether to allow bacterial invasion to proceed. In the past few years, several plant mutants affected in IT formation and growth have been isolated, and their further characterization will likely shed light on some of the mechanisms that the host uses to create and control the growth of this structure (30,91,106,163,176,189).

Rhizobial invasion of the host nodule via the IT is strongly influenced by a complex variety of bacterial polysaccharides in addition to NF, including secreted EPSs and K-antigens, secreted and periplasmic cyclic β glucans, and the outer membrane-localized LPSs (14,57, 78,155,157). Similar to NF, several of these molecules exert their effects on symbiosis in a structurally dependent manner, arguing that they may function as signals between invading bacteria and their host. In fact, recent evidence suggests that the exopolysaccharide succinoglycan may help further define species-specificity in addition to NF (153). A shared and outstanding question regarding these bacterial polysaccharides is their potential role in

modulating a plant defense response to bacterial invasion. However, definitive proof for such possibilities awaits the identification and characterization of specific host receptors.

Exopolysaccharide

The biosynthesis of rhizobial EPS and its regulation have been most extensively studied with regard to the *S. meliloti* macromolecule referred to as succinoglycan, or EPS I, (Figure 2*c*) (15, 78, 155). *S. meliloti* succinoglycan is secreted as a polymer of repeating octasaccharide subunits (one galactose and seven glucose residues) modified with succinyl, acetyl, and pyruvyl substituents (Figure 2*c*). Polymerization of the succinoglycan monomer results in secretion of either low molecular weight (LMW) forms (monomers, dimers, and trimers) or high molecular weight (HMW) forms (polymers of several hundred subunits).

Succinoglycan plays a critical role in the *S. meliloti* symbiosis with *Medicago* hosts. Succinoglycan-deficient mutants elicit nodule organogenesis by virtue of NF signaling (86), but these aberrantly small nodules are devoid of bacteria and therefore incapable of nitrogen fixation (99). These rhizobial mutants are compromised for host invasion to the extent that IT formation proceeds from only 10% of bacterially colonized root hairs and the ITs that do form terminate prematurely before they reach the nodule primordium (27). While EPSs play a passive role in protecting the bacterium from host-derived stresses within the IT (36), they are also thought to perform a signaling function from rhizobia to their host (78,79). Part of the evidence for this is based on the observation that LMW forms of succinoglycan are more effective at promoting symbiosis than HMW forms (10,169,182). Moreover, an *exoH* mutant is unable to succinylate the succinoglycan monomer and therefore produces predominantly HMW succinoglycan (98); since this mutant is also severely compromised for symbiosis a merely protective role is not sufficient to explain all of the requirements for succinoglycan in host invasion.

Increasingly, the evidence available suggests that bacterial EPSs play a role in modulating the host defense response to bacterial invasion. For example, the premature termination of bacterial infection observed with EPS-deficient mutants is associated with symptoms of a host defense response and, in particular, the production of antimicrobial phenolics and phytoalexins (119, 126). To test the hypothesis that succinoglycan promotes symbiosis as a signaling molecule, a global transcriptome analysis was performed on *M. truncatula* plants inoculated with succinoglycan-deficient *S. meliloti* at a time point just prior to IT formation (3 days postinoculation) (79). Thus, this experiment identified host genes differentially regulated in response to EPS production, rather than IT failure per se, and the largest group of genes upregulated during an EPS-deficient interaction encode putative plant defense proteins (79). As mentioned above, *M. truncatula* defense genes are upregulated 1 h postinoculation with wild type *S. meliloti*; however, expression of this same class of genes decreases by 6 h postinoculation and remains low for at least 3 days (105). Taken together, these observations suggest that a primary consequence of EPS production is the suppression of a potentially lethal host defense response, and in the absence of EPS, this unproductive response may cause a block in IT formation (27).

Insight into how *S. meliloti* regulates production and modification of exopolysaccharides is therefore important to our understanding of the physiological requirements for host invasion. An increasingly large number of regulators modulate succinoglycan production *ex planta*, including ExoS, ExoR MucR, SyrA, and the more recently identified CbrA (62,155).

The first regulators of succinoglycan to be identified were the ExoS two-component histidine kinase and ExoR. ExoS is an essential gene in *S. meliloti* and forms a two-component signal transduction pathway with the response regulator ChvI, which is also essential and functions as a DNA-binding transcription factor (26). ExoS phosphorylates ChvI directly and thereby

promotes increased *exo*/*exs* expression and greater succinoglycan production (26). ExoR has no homology to any known regulators, however a null mutation alters the transcription of several *exo* biosynthetic genes (138). Early observations suggested a genetic link between *exoR* and *exoS*: extragenic suppressors of an *exoR* null mutant symbiosis defect were mapped near *exoS* and these suppressors displayed a concomitant change in *exo* gene transcription (125). More recent evidence indicates that ExoR is a periplasmic protein that functions upstream of the ExoS/ChvI two-component pathway and is hypothesized to directly repress ExoS kinase activity by binding its periplasmic sensing domain (183). An outstanding question remains as to the nature of the stimulus perceived by ExoS and/or ExoR.

The two-component histidine kinase CbrA has also been identified as a regulator of succinoglycan production. Unlike the overproduction of wild-type forms of succinoglycan in *exoS* and *exoR* mutants (46), a *cbrA* mutant appears to overproduce predominantly LMW forms of succinoglycan, and this phenotype is correlated with increased transcription of several *exo* genes involved in LMW succinoglycan biosynthesis, including *exoH*, *exoK*, and *exoT* (62). Moreover, the *cbrA* mutation leads to increased expression of additional genes involved in promoting bacterial IT invasion, including the *ndvA* transporter of cyclic β glycans (described below) and the *sinI* regulator of galactoglucan (EPS II) production (61). Thus, it was proposed that CbrA coordinates multiple aspects of bacterial physiology to promote bacterial invasion of the nodule, and that the physiology of the *cbrA* mutant is optimized for this process. Given that CbrA contains at least one PAS domain (62), a motif that commonly monitors redox changes, CbrA may coordinate bacterial physiology in response to the high redox environment of the IT. However, since the *cbrA* mutant remains defective for symbiosis despite a physiology optimized for IT invasion (61), it appears that CbrA plays an additional role during subsequent bacteroid differentiation, as discussed below.

Cyclic β Glucan

Rhizobia generally produce β-(1,2)-glucans that are macrocyclic and unbranched polymers of glucose, containing anywhere from 17 to 40 residues depending on the rhizobial strain (157). The synthesis of cyclic β glucan is dependent on a glycosyltransferase encoded by *ndvB* (called *cgs* in *Mesorhizobium loti*) and its secretion is dependent on the ABC-type inner membrane transporter *ndvA*. *Bradyrhizobium* species are an exception and produce branched macrocyclic glucans that contain both β-(1,3) and β-(1,6) glycosidic bonds catalyzed by glycosytransferases encoded by *ndvB* and *ndvC*. The chemical characteristics of cyclic β glucans can be modified by the addition of phosphocholine, sn-1-phosphoglycerol, succinic and methylmalonic acid substituents. Disruption of *ndvB* (*cgs*) in *S. meliloti* (*M. loti*) blocks symbiosis at the stage of bacterial attachment to root hairs and IT invasion and thereby results in the formation of aberrantly small and empty nodules (34,48). In contrast, a *B. japonicum ndvB* mutant is able to elicit normal nodule development and invade host tissues, although the resulting nodules do not fix nitrogen (47).

Like succinoglycan, the cyclic β glucans may play a role in modulating a host defense response to bacterial invasion. Specifically, *M. loti* cyclic β glycans are required to suppress high-level production of antimicrobial phytoalexins during symbiotic development with *L. japonicus* (35). Many host defense response genes are induced in a mature nodule during a wild-type symbiosis (29,90), perhaps to provide the nutrient-rich nodule with a defense against parasites. These same genes are generally expressed at decreased levels during the ineffective symbiosis of the *cgs* mutant, with the striking exception of highly induced *PAL* expression (35). *PAL* encodes an enzyme predicted to participate in the synthesis of antimicrobial phenolic compounds, and consistent with increased *PAL* expression during the *cgs* mutant symbiosis, *L. japonicus* nodules accumulate phenolic compounds to a greater extent than is observed during a wild-type symbiosis (35). Purified *B. japonicum* cyclic β glucans are able to block a

host defense response to fungal elicitors in the determinate legume *G. max* and in a structurally dependent manner (17), further suggesting these polysaccharides may be able to prevent a host defense response during rhizobial invasion.

BACTERIAL REQUIREMENTS FOR INTRACELLULAR COLONIZATION

Lipopolysaccharide

Throughout symbiotic development the *S. meliloti* cell surface is in intimate association with its host but this is particularly true of the microsymbiont within the symbiosome. Not surprisingly then, the bacterial cell surface plays an important role in promoting rhizobial intracellular adaptation, including the lipopolysaccharide (LPS) component of the gram-negative outer membrane (14,83,157). LPS is a complex macromolecule composed of a lipid A membrane anchor and an oligosaccharide core, which can be further modified by the addition of a variable O-antigen polysaccharide (135). Generally, host perception of LPS from pathogenic bacteria plays a significant role in defense responses to invasion via the innate immune system (130). It has therefore been of great interest to understand the specific role that rhizobial LPS plays in symbiotic development. Bacteroid LPS has increased hydrophobicity compared to that of free-living bacteria, suggesting there are LPS modifications *in planta* that may contribute to symbiosis (38,84).

Rhizobia produce a lipid A with unique structural characteristics that distinguish it from the potent *E. coli* innate defense elicitor endotoxin (135), although there is variation in certain elements among the rhizobia (14,83). For instance, most rhizobia produce lipid A species lacking either one or both of the 1- and 4′-phosphate groups present on the β-(1,6)-glucosamine disaccharide of *E. coli; Sinorhizobium* lipid A is an exception to this trend as it is bisphosphorylated (Figure 2*d*). In addition, rhizobial lipid A moieties are most often modified with a secondary N-linked acyloxyacyl residue composed of a C28 or C30 Very-Long-Chain Fatty Acid (VLCFA) that has the capacity to span the entire lipid bilayer of the outer membrane (Figure 2*d*). This particular modification has generated much interest as a structural feature that could shield rhizobial LPS from recognition by the host innate immune system and thereby promote rhizobial invasion and persistence. In fact, the presence of VLCFA modified lipid A has been observed in several related bacteria that establish persistent host infections, including all rhizobia, agrobacteria, and brucella that have been analyzed.

The *acpXL* and *lpsXL* genes encode an acyl carrier protein and an acyl transferase, respectively, that together catalyze the VLCFA secondary acylation of lipid A in rhizobia. Rhizobial *acpXL* and *lpsXL* mutants completely lack the VLCFA-modified lipid A during free-living growth but remain effective at establishing a nitrogen-fixing symbiosis (53,150,175), although bacteroid development is mildly perturbed and leads to decreased nitrogen-fixation (173). This weak symbiosis phenotype is likely explained by the recent discovery that *acpXL* mutants of *R. leguminosarum* b.v. *viciae* form bacteroids that actually contain VLCFA-modified lipid A, indicating that they express an alternative system for VLCFA modification in planta (174); thus, the role that the VLCFA modification plays in symbiosis remains to be determined.

Alteration of the LPS carbohydrate content, in either the core or the O-antigen, has an aberrant effect in a variety of symbioses (83). For example, an *S. meliloti lpsB* mutant has a dramatically altered LPS core and is incapable of establishing a chronic host infection (21). While the mutant displays normal host IT invasion and is taken up into the host cell cytoplasm, it undergoes rapid senescence and is degraded within the symbiosome compartment, suggesting LPS plays a critical role in rhizobial adaptation and persistence within the particular environment of the host cell cytoplasm (21).

The precise function of LPS in promoting symbiosis remains unclear (14). Defects in LPS can sensitize bacteria to membrane-disrupting agents and antimicrobial peptides so that it may provide a protective barrier against environmental stress and host defense responses. However, there are indications that *S. meliloti* LPS may also play an active role by suppressing the release of ROS (1,148). Specifically, *M. truncatula* tissue culture cells respond to yeast elicitors with an oxidative burst and the increased expression of defense genes involved in plant secondary metabolism, like *PAL*, and cell wall metabolism (1,164). When tissue culture cells are exposed to elicitor in the presence of *S. meliloti* lipid A, this LPS component is capable of suppressing the oxidative burst and dampening the plant transcriptional response (148,164), indicating an interaction between rhizobial LPS and its host could suppress any potential immune response to intracellular bacteria. This could be particularly important for bacteria within the symbiosome as they no longer express genes for the biosynthesis of succinoglycan (9), which appears to dampen a potential plant defense response to bacteria within the IT (78,79). Lipid A moieties isolated from the related intracellular mammalian pathogen *Brucella abortus*, which also undergo VLCFA modification, are only weak elicitors of an innate immune response in mouse macrophages (93,94). Perhaps these related alphaproteobacteria share some of the strategies that rhizobia use to evade detection by the innate immune system during chronic infection.

BacA

The *bacA* gene encodes an inner membrane protein that plays an essential role in the early stages of *S. meliloti* bacteroid development (64). With a *bacA* mutant, the early steps in symbiosis, such as formation and development of infection threads, proceed as efficiently as with wild type but the mutant lyses shortly after being endocytosed into the cytoplasm of plant cells. BacA function is also essential for the chronic infection of *B. abortus* (100), a mammalian pathogen that can survive and replicate in host macrophages, highlighting the broad importance of BacA function to chronic bacterial persistence during intracellular infection (140).

Generally, the loss of *bacA* function has been associated with an increased resistance to certain antimicrobial peptides (74,96,113). In *E. coli*, deletion of the *bacA* isofunctional homolog, *sbmA*, produces increased resistance to microcins B17 and J25, bleomycin, and Bac7, which is an antimicrobial peptide of mammalian origin (96,113). Similarly, the *bacA* mutants of *S. meliloti* and *B. abortus* show increased resistance to bleomycin relative to the wild type (74, 100). Thus, BacA may play a role in the transport of modified peptides across the inner membrane (64). In fact, it was proposed that BacA functions as an importer for host-derived peptide(s) that promote bacteroid differentiation (114). Specifically, it was suggested that bacterial uptake of Nodule-specific Cysteine-Rich (NCR) peptides produced by indeterminate legumes could trigger the terminal differentiation of bacteroids (3,114), analogous to the role defensins play in suppressing the proliferation of bacterial pathogens (190).

In *S. meliloti* and *B. abortus*, BacA also affects the VLCFA modification of the lipid A component of LPS (52,54). BacA has homology to the transmembrane domain of eukaryotic ATP-binding cassette (ABC) transporters of the ABCD family (52). ABCD transporters function in the peroxisome and this includes the human adrenoleukodystrophy protein (hALDP) that is thought to transport activated VL-CFAs across the peroxisomal membrane (181). Approximately 50% of lipid A moieties isolated from *S. meliloti bacA* mutants (as well as *B. abortus*) lack VLCFAs (52). Thus, it was suggested that BacA may export VLCFAs from the cytoplasm and across the inner membrane, and perhaps the lack of VLCFA-modified lipid A causes the defects in chronic infection observed with *bacA* mutants (52). As mentioned above, rhizobial *acpXL* and *lpxXL* mutants completely lack VLCFA-modified lipid A during free-living growth and are able to establish a successful symbiosis despite bacteroid morphological abnormalities (53,150,173,175). However, at least *R. leguminosarum acpXL*

bacteroids contain VLCFA-modified lipid A in planta (174), indicating an alternative system for VLCFA modification exists that may require BacA function.

It remains to be determined whether BacA is directly responsible for either one or both of the proposed transport reactions. *S. meliloti* strains carrying 12 site-directed mutations in *bacA* have phenotypes intermediate to the wild-type and *bacA* null mutant (101), consistent with a role for BacA in multiple, nonoverlapping functions. Moreover, it was reported that functional SbmA is required for full efficiency of the tetracycline exporter TetA in *E. coli* (42), suggesting that BacA function could similarly affect the activity of other membrane proteins. Future studies that reconstitute BacA into liposomes, followed by detailed transport assays, will be needed to elucidate its precise function.

DEVELOPMENTAL REGULATION OF THE CELL CYCLE

Symbiotic regulation of the plant cell cycle and the role of endoreduplication in nodule development has been studied extensively (56,122). In contrast, an understanding of how the bacterial cell cycle may be regulated during symbiosis is just beginning to emerge. Bacteroids within determinate hosts have the ability to dedifferentiate into free-living bacteria once they are released from a senescent nodule (114). In contrast, at least some rhizobia that form a symbiosis with indeterminate legumes undergo a terminal differentiation program that precludes viability outside the host cell cytoplasm (114). Whether a given rhizobial species undergoes terminal differentiation appears to be a decision controlled by the legume host (114).

During free-living growth, and presumably within the IT, *S. meliloti* grows as a rod-shaped bacterium with no greater than a 2N complement of its genome (Figure 3*a*,*b*) (114), which implies that these bacteria initiate DNA replication only once per cell cycle. In contrast, one remarkable aspect of terminal bacteroid differentiation is a branched cell morphology that is accompanied by several rounds of genomic endoreduplication (Figure 3*c*) (114). The correlation between bacteroid endoreduplication and the inability to dedifferentiate and resume growth outside the host suggests the act of endoreduplication may be a defining event in terminal bacteroid differentiation. The indeterminate symbiosis therefore represents a novel bacterial cell cycle event, i.e., endoreduplication, which may play an important role in either intracellular persistence or efficient nitrogen fixation. Taken together, these observations imply that the *S. meliloti* cell cycle has at least three branch points subject to in planta regulation (Figure 3*d*), and it will be of great interest to understand how the cell decides which path to choose under different host conditions.

While regulatory aspects of the *S. meliloti* cell cycle have been little studied, that of the related *Caulobacter crescentus* alphaproteobacterium has been dissected in great detail and thereby serves as a powerful model on which to base future studies in *S. meliloti*. Briefly, in *C. crescentus* the CtrA response regulator collaborates with the DnaA replication initiator and GcrA transcription factor to globally control cell cycle progression (154). Throughout the cell cycle, CtrA concentrations are strictly regulated, and this plays a critical role in mediating cell cycle progression. Additionally, an elaborate two-component signal transduction pathway containing the essential response regulator DivK regulates the concentration of active CtrA-P (18). Through this multi-layered regulation of active CtrA-P concentrations, *C. crescentus* limits the initiation of DNA replication to once per cell cycle (Figure 4*a*), in contrast with *E. coli* and other fast-growing bacteria. The coordination between DNA replication and cell division that allows only one replication initiation event per cell cycle is also observed in *S. meliloti* (114).

C. crescentus undergoes an asymmetric cell division during each cell cycle for the purpose of nutrient adaptation (Figure 4*b*), and the DivJ and PleC two-component histidine kinase sensors mediate this asymmetry. Other alphaproteobacteria also undergo asymmetric cell division and have orthologs of *C. crescentus* cell cycle regulators present in their genomes (70), including *S. meliloti* (Figure 4*c*), *B. abortus*, and *Agrobacterium tumefaciens*. CbrA has strong homology in its C-terminal kinase domain to the DivJ and PleC kinases of DivK (70), suggesting that its primary function may be to regulate cell cycle progression in *S. meliloti* via regulation of DivK phosphorylation, as was observed for its *B. abortus* ortholog PdhS (71). In fact, during free-living growth the *cbrA* mutant displays a filamentous, branched morphology that coincides with a greater than 2N genomic content, indicating the mutant is unable to coordinate DNA replication with cell division (K.E. Gibson & G.C. Walker, unpublished). Perhaps CbrA plays a role in modifying the cell cycle program during symbiosis and loss of this function is primarily responsible for the in planta defects of the *cbrA* mutant. If this is the case, it will be of interest to determine how CbrA coordinates cell cycle progression with cell envelope physiology and succinoglycan production. It is possible that at least some of the *cbrA* mutant phenotypes are an indirect consequence of altered cell cycle progression given that multiple cell surface characteristics are cell cycle regulated in *C. crescentus*.

The process of endoreduplication undoubtedly creates an intense demand for dNTPs within the developing bacteroid. The dNTPs required for DNA metabolism in all organisms are synthesized by the enzyme ribonucletide reductase (RNR) (78). *S. meliloti* has only one RNR encoded in its genome, NrdJ, and this is a vitamin B_{12}-dependent enzyme (33). As a Class II RNR that is both oxygen-independent and oxygen-insensitive (80), this enzyme likely provides a key adaptation for rhizobial persistence within the microaerobic environment of the host cell cytoplasm. The B_{12} biosynthetic enzyme BluB, which catalyzes formation of the lower ligand 5,6-dimethylbenzimidazole, was fortuitously found to be required for symbiosis between *S. meliloti* and *M. sativa* based on its involvement in succinoglycan biosynthesis (22,162). A *bluB* mutant appears able to infect the host via normal IT growth, suggesting that any alteration to the succinoglycan does not affect invasion; however, bacteroids are not observed within the host cell cytoplasm and the nodules that develop are unable to fix nitrogen (22). Thus, BluB function in B_{12} biosynthesis is necessary for symbiosis due to the requirement for a B_{12}-dependent enzyme(s), for which NrdJ is one possible candidate.

During the indeterminate symbiosis between *Mesorhizobium huakuii* and *Astragalus sinicus*, bacterial DNA replication is limited to those bacteria associated with the meristem in Infection Zone II and Interzone II/III (89). This observation suggests that, at least in some rhizobia, DNA replication is permanently blocked once the bacteroid completes endoreduplication. Bacterial genes involved in DNA repair are induced within mature nodules (9), suggesting that DNA integrity is maintained within bacteroids in the absence of DNA replication. Importantly, these DNA repair genes include several that encode nonhomologous end-joining (NHEJ) proteins involved in double strand break repair (88), a process also utilized by terminally differentiated cells in higher eukaryotes.

Little is currently known regarding rhizobial cell division during symbiosis, although the altered morphology of bacteroids implies an underlying regulation of this process. Several *S. meliloti* genes involved in cell division have been characterized, for instance *ftsZ1* and *ftsZ2* (108,110,111), as well as *minCDE* (28). Blocking the process of cell division via over-expression of *ftsZ1* or *minCD* causes altered cell morphology during free-living growth that is reminiscent of the branched and filamentous bacteroid (28,95). Treatment of *S. meliloti* with DNA-damaging agents, which impinge on DNA replication and cell division in other bacteria, also inhibits cell division and results in branched cell morphology (95). Although the mechanisms that underlie these changes in cell morphology and how they relate to bacteroid

differentiation are still unknown, *ftsZ1* and *ftsZ2* expression is decreased in bacteroids consistent with a block in cell division after differentiation (9,13).

NUTRIENT EXCHANGE

Invading bacteria within the IT are ultimately endocytosed by postmitotic G_0 endoploid cells and remain encapsulated within a modified plasma membrane called the peribacteroid membrane (PBM) (Figure 1*d*, *f*). The symbiosome, consisting of the PBM and its microsymbiont, is thereby formed and within this PBM-bound compartment bacteria establish a persistent, or chronic, infection of the host. In this manner, a single host cell will ultimately house hundreds of bacteria. Only after bacteria have gained access to the host cell cytoplasm do they differentiate into the morphologically distinct bacteroid form that is capable of nitrogen fixation. The in planta differentiation of rhizobia involves significant metabolic changes that promote adaptation and nitrogen fixation (121, 131). For example, rhizobia undertake respiratory chain modifications that allow energy utilization under microaerobic conditions, repress glycolysis genes, and activate C_4-dicarboxylic acid utilization pathways for carbon metabolism. Induction of nitrogenase gene expression is triggered in a FixJ-dependent manner by the microaerobic conditions of the host cell cytoplasm (55), which is in turn critical for the activity of this oxygen-sensitive enzyme (45, 92). In addition to genes required for nitrogenase activity, FixJ is responsible for induction of nearly all the bacterial genes whose expression increases in mature nodules (9), indicating that the oxygen-sensing two-component pair FixL and FixJ plays a profound role in regulating bacteroid physiology. In contrast, host gene expression in nodules colonized by the rhizobial *fixJ* mutant is largely indistinguishable from nodules colonized by wild-type rhizobia, suggesting that nodule morphogenesis and bacterial invasion contribute more to host gene regulation than does nitrogen fixation (9).

Creation and maintenance of the host microaerobic environment is dependent on structural aspects of the nodule that form an oxygen diffusion barrier in combination with high expression levels of plant leghemoglobin, which can be 25% of total soluble protein in a nodule and helps limit the concentration of free oxygen to 3–22 nM (124,167,186). The host supports high nitrogenase activity by providing bacteroids with a constant flux of O_2 for aerobic respiration and with energy in the form of C_4-dicarboxylic acids derived from the photosynthate sucrose (102,141,143). The metabolic product of the nitrogenase enzyme reaction is ammonia, and this appears to be provided to the host both directly and indirectly through its incorporation into alanine by the bacterial enzyme alanine dehydrogenase (2). Generally, nitrogen secreted from the bacteroid is assimilated by the host through its incorporation into the amino acids glutamine and glutamate by the enzymes glutamine synthetase (GS) and glutamate synthase (GOGAT), respectively (185). In determinate nodules, the large bacterially infected cells are interspersed with small non-infected cells that appear to be specialized for further nitrogen assimilation, involving conversion of amino acids (glutamine and arginine) into either ureides or amides that are exported from the nodule to the rest of the plant.

HOST SANCTIONS ON SYMBIOTIC RHIZOBIA

From an evolutionary point of view, why do rhizobial bacteria maintain the large number of genes required for mutualism with their legume hosts (44)? This question is particularly relevant in light of the recent observation that bacteroids within indeterminate nodules are terminally differentiated and unable to give rise to progeny (114). However, even a mature indeterminate nodule in the soil can contain anywhere from 10^5–10^{10} clonally related bacteria that are located within the invasion zone (Zone II; Figure 1*a*) and have not yet differentiated. Thus, a single symbiotic rhizobium is predicted to have greater fitness if it successfully colonizes a nodule than its nonsymbiotic cousin residing in the soil where growth can be severely limited by nutrient availability.

While there appears to be a fitness gain for rhizobia able to invade the nodule, it is also clear that the host has evolved mechanisms that prevent nonfixing rhizobia from parasitizing the legume nodule for energy. While the host controls the infection process and nodule morphology, it is the microsymbiont that largely dictates the efficiency of nitrogen fixation. Mathematical modeling suggests that if legumes treat fixing and nonfixing rhizobial strains within the nodule similarly, then nonfixing rhizobia would quickly outcompete nitrogen fixers (184). Perhaps for this reason, the host imposes effective sanctions on nonfixing rhizobial cheaters within the nodule (85,184). So far, host sanctions have been found to take the form of severe O_2 limitation to nonfixing rhizobia within the nodule, which restricts bacterial growth and viability. Thus, the legume host is capable of imposing selective pressure on rhizobia that may affect the evolution of bacterial populations in favor of nitrogen fixers (44).

SUMMARY POINTS

1. There tends to be strict species-specificity between legumes and their compatible symbionts, and this specificity is defined in part by NF structure. NF elicits changes in host physiology and gene expression that lead to nodule development by virtue of a NF-perception pathway involving LysM receptor-like kinases. The host specificity engendered by NF structure is dependent on the extracellular LysM domain, which likely binds NF directly. Initial IT formation and its continued growth also has NF requirements and in fact displays a greater stringency for chemical modification of the NF backbone than do earlier physiological responses.
2. NF elicits two temporally and genetically separable effects on host ROS production. During initial stages of symbiosis, NF is responsible for reducing ROS production and this promotes early morphological responses in root hairs that initiate the process of rhizobial invasion. During subsequent nodule development, a host-derived ROS efflux produced in response to NF plays a positive role in promoting bacterial IT invasion, possibly by modifying the cell wall of the IT or by providing a redox signal to bacteria that elicits IT-specific physiological adaptations.
3. A variety of rhizobial cell-surface and secreted polysaccharides appear to function as signals from the bacterium to its host. In particular, transcriptome analyses of host responses to polysaccharide-deficient bacterial mutants suggest that at least one role for these macromolecules is to suppress the expression of certain host defense response genes, such as *PAL*, that are involved in the production of antimicrobial compounds. Moreover, purified rhizobial LPS and cyclic β glucan are capable of inhibiting the plant defense response to fungal elicitor in a tissue culture setting.
4. *S. meliloti* typically initiates DNA replication only once per cell cycle during free-living growth and presumably within the host IT. However, bacteria that colonize the host cell cytoplasm undergo the novel process of endoreduplication as it differentiates into a bacteroid. Ultimately, this bacteroid becomes terminally differentiated such that no further DNA replication or cellular growth is observed and its viability becomes strictly limited to the intracellular environment of the host. These observations imply that the *S. meliloti* cell cycle has at least three unique branch points that are subject to in planta regulation.

Acknowledgments

We apologize to those investigators whose work we could not discuss owing to space limitations. We thank members of the Walker lab for many thoughtful discussions and for critical reading of the manuscript. This work was supported

by the National Institute of Health grant GM31010 (to G.C.W.), National Cancer Institute (NCI) grant CA21615-27 (to G.C.W.) and JSPS Postdoctoral Fellowships for Research Abroad (to H.K.). G.C.W. is an American Cancer Society Research Professor.

Glossary

Rhizobia
: gram-negative alphaproteobacteria that engage in symbiosis with legumes and fix nitrogen

Horizontal gene transfer
: incorporation of genetic material from an unrelated organism (also called lateral gene transfer)

Chromosomal island
: section within a genome having evidence of horizontal origins

NF
: nodulation (Nod) factor

EPS
: exopolysaccharide

Rhizosphere
: local soil environment surrounding plant roots

Synteny
: preservation of gene order on chromosomes of related species

Meristem
: undifferentiated plant cells undergoing cell growth and division

Endoreduplication
: repeated genomic replication without cytokinesis

Endoploid
: cells that have undergone endoreduplication

IT
: infection thread

Indeterminate nodule
: nodule with a persistent meristem and a heterogenous plant and bacterial developing cell population

Determinate nodule
: nodule with a temporary meristem and a relatively homogenous plant and bacterial developing cell population

Mitogen
: a substance that elicits cell division

Transcriptome
: complete set mRNAs expressed in a cell or cell population

LPS
: lipopolysaccharide

LysM

lysin motif

ROS
reactive oxygen species

RNS
reactive nitrogen species

VLCFA
very-long-chain fatty acid

PBM
peribacteroid membrane

LITERATURE CITED

1. Albus U, Baier R, Holst O, Puhler A, Niehaus K. Suppression of an elicitor-induced oxidative burst in *Medicago sativa* cell-cultures by *Sinorhizobium meliloti* lipopolysaccharides. New Phytol 2001;151:597–606.
2. Allaway D, Lodwig EM, Crompton LA, Wood M, Parsons R, et al. Identification of alanine dehydrogenase and its role in mixed secretion of ammonium and alanine by pea bacteroids. Mol Microbiol 2000;36:508–15. [PubMed: 10792736]
3. Alunni B, Kevei Z, Redondo-Nieto M, Kondorosi A, Mergaert P, Kondorosi E. Genomic organization and evolutionary insights on GRP and NCR genes, two large nodule-specific gene families in *Medicago truncatula*. Mol Plant Microbe Interact 2007;20:1138–48. [PubMed: 17849716]
4. Ardissone S, Frendo P, Laurenti E, Jantschko W, Obinger C, et al. Purification and physical-chemical characterization of the three hydroperoxidases from the symbiotic bacterium *Sinorhizobium meliloti*. Biochemistry 2004;43:12692–99. [PubMed: 15449959]
5. Ardourel M, Demont N, Debelle F, Maillet F, de Billy F, et al. *Rhizobium meliloti* lipooligosaccharide nodulation factors: different structural requirements for bacterial entry into target root hair cells and induction of plant symbiotic developmental responses. Plant Cell 1994;6:1357–74. [PubMed: 7994171]The structural requirements for *S. meliloti* NF are more stringent for IT growth than earlier root hair responses.
6. Barcellos FG, Menna P, da Silva Batista JS, Hungria M. Evidence of horizontal transfer of symbiotic genes from a *Bradyrhizobium japonicum* inoculant strain to indigenous diazotrophs *Sinorhizobium* (Ensifer) *fredii* and *Bradyrhizobium elkanii* in a Brazilian savannah soil. Appl Environ Microbiol 2007;73:2635–43. [PubMed: 17308185]
7. Barloy-Hubler F, Cheron A, Hellegouarch A, Galibert F. Smc01944, a secreted peroxidase induced by oxidative stresses in *Sinorhizobium meliloti* 1021. Microbiology 2004;150:657–64. [PubMed: 14993315]
8. Barnett MJ, Fisher RF, Jones T, Komp C, Abola AP, et al. Nucleotide sequence and predicted functions of the entire *Sinorhizobium meliloti* pSymA megaplasmid. Proc Natl Acad Sci USA 2001;98:9883–88. [PubMed: 11481432]
9. Barnett MJ, Toman CJ, Fisher RF, Long SR. A dual-genome Symbiosis Chip for coordinate study of signal exchange and development in a prokaryote-host interaction. Proc Natl Acad Sci USA 2004;101:16636–41. [PubMed: 15542588]A transcriptome analysis of the host and its symbiont in tandem.
10. Battisti L, Lara JC, Leigh JA. Specific oligosaccharide form of the *Rhizobium meliloti* exopolysaccharide promotes nodule invasion in alfalfa. Proc Natl Acad Sci USA 1992;89:5625–29. [PubMed: 1608972]
11. Batut J, Andersson SG, O'Callaghan D. The evolution of chronic infection strategies in the alpha-proteobacteria. Nat Rev Microbiol 2004;2:933–45. [PubMed: 15550939]
12. Baudouin E, Pieuchot L, Engler G, Pauly N, Puppo A. Nitric oxide is formed in *Medicago truncatula–Sinorhizobium meliloti* functional nodules. Mol Plant Microbe Interact 2006;19:970–75. [PubMed: 16941901]

13. Becker A, Berges H, Krol E, Bruand C, Ruberg S, et al. Global changes in gene expression in *Sinorhizobium meliloti* 1021 under microoxic and symbiotic conditions. Mol Plant Microbe Interact 2004;17:292–303. [PubMed: 15000396]

14. Becker A, Fraysse N, Sharypova L. Recent advances in studies on structure and symbiosis-related function of rhizobial K-antigens and lipopolysaccharides. Mol Plant Microbe Interact 2005;18:899–905. [PubMed: 16167760]

15. Becker A, Ruberg S, Baumgarth B, Bertram-Drogatz PA, Quester I, Puhler A. Regulation of succinoglycan and galactoglucan biosynthesis in *Sinorhizobium meliloti*. J Mol Microbiol Biotechnol 2002;4:187–90. [PubMed: 11931545]

16. Bender GL, Nayudu M, Le Strange KK, Rolfe BG. The *nodD1* gene from *Rhizobium* strain NGR234 is a key determinant in the extension of host range to the nonlegume *Parasponia*. Mol Plant-Microbe Interact 1988;1:259–66.

17. Bhagwat AA, Mithofer A, Pfeffer PE, Kraus C, Spickers N, et al. Further studies of the role of cyclic beta-glucans in symbiosis. An NdvC mutant of *Bradyrhizobium japonicum* synthesizes cyclodecakis-(1–>3)-beta-glucosyl. Plant Physiol 1999;119:1057–64. [PubMed: 10069844]

18. Biondi EG, Reisinger SJ, Skerker JM, Arif M, Perchuk BS, et al. Regulation of the bacterial cell cycle by an integrated genetic circuit. Nature 2006;444:899–904. [PubMed: 17136100]

19. Boussau B, Karlberg EO, Frank AC, Legault BA, Andersson SG. Computational inference of scenarios for alpha-proteobacterial genome evolution. Proc Natl Acad Sci USA 2004;101:9722–27. [PubMed: 15210995]

20. Caetano-Anolles G, Crist-Estes DK, Bauer WD. Chemotaxis of *Rhizobium meliloti* to the plant flavone luteolin requires functional nodulation genes. J Bacteriol 1988;170:3164–69. [PubMed: 3384804]

21. Campbell GR, Reuhs BL, Walker GC. Chronic intracellular infection of alfalfa nodules by *Sinorhizobium meliloti* requires correct lipopolysaccharide core. Proc Natl Acad Sci USA 2002;99:3938–43. [PubMed: 11904442]

22. Campbell GR, Taga ME, Mistry K, Lloret J, Anderson PJ, et al. *Sinorhizobium meliloti bluB* is necessary for production of 5,6-dimethylbenzimidazole, the lower ligand of B_{12}. Proc Natl Acad Sci USA 2006;103:4634–39. [PubMed: 16537439]

23. Capela D, Carrere S, Batut J. Transcriptome-based identification of the *Sinorhizobium meliloti* NodD1 regulon. Appl Environ Microbiol 2005;71:4910–13. [PubMed: 16085895]

24. Catoira R, Timmers AC, Maillet F, Galera C, Penmetsa RV, et al. The *HCL* gene of *Medicago truncatula* controls Rhizobium-induced root hair curling. Development 2001;128:1507–18. [PubMed: 11290290]

25. Chen WM, Moulin L, Bontemps C, Vandamme P, Bena G, Boivin-Masson C. Legume symbiotic nitrogen fixation by beta-proteobacteria is widespread in nature. J Bacteriol 2003;185:7266–72. [PubMed: 14645288]The nitrogen-fixing legume symbiosis extends beyond the alphaproteobacteria.

26. Cheng HP, Walker GC. Succinoglycan production by *Rhizobium meliloti* is regulated through the ExoS-ChvI two-component regulatory system. J Bacteriol 1998;180:20–26. [PubMed: 9422587]

27. Cheng HP, Walker GC. Succinoglycan is required for initiation and elongation of infection threads during nodulation of alfalfa by *Rhizobium meliloti*. J Bacteriol 1998;180:5183–91. [PubMed: 9748453]An analysis of IT formation and growth using GFP-expressing bacteria.

28. Cheng J, Sibley CD, Zaheer R, Finan TM. A *Sinorhizobium meliloti minE* mutant has an altered morphology and exhibits defects in legume symbiosis. Microbiology 2007;153:375–87. [PubMed: 17259609]

29. Colebatch G, Desbrosses G, Ott T, Krusell L, Montanari O, et al. Global changes in transcription orchestrate metabolic differentiation during symbiotic nitrogen fixation in *Lotus japonicus*. Plant J 2004;39:487–512. [PubMed: 15272870]

30. Combier JP, Vernie T, de Billy F, El Yahyaoui F, Mathis R, Gamas P. The MtMMPL1 early nodulin is a novel member of the matrix metalloendoproteinase family with a role in *Medicago truncatula* infection by *Sinorhizobium meliloti*. Plant Physiol 2007;144:703–16. [PubMed: 17293436]

31. Cook D, Dreyer D, Bonnet D, Howell M, Nony E, VandenBosch K. Transient induction of a peroxidase gene in *Medicago truncatula* precedes infection by *Rhizobium meliloti*. Plant Cell 1995;7:43–55. [PubMed: 7696879]

32. Cooper JE. Early interactions between legumes and rhizobia: disclosing complexity in a molecular dialogue. J Appl Microbiol 2007;103:1355–65. [PubMed: 17953546]
33. Cowles JR, Evans HJ, Russell SA. B_{12} Coenzyme-dependent ribonucleotide reductase in Rhizobium species and the effects of cobalt deficiency on the activity of the enzyme. J Bacteriol 1969;97:1460–65. [PubMed: 5780513]
34. D'Antuono AL, Casabuono A, Couto A, Ugalde RA, Lepek VC. Nodule development induced by *Mesorhizobium loti* mutant strains affected in polysaccharide synthesis. Mol Plant Microbe Interact 2005;18:446–57. [PubMed: 15915643]
35. D'Antuono AL, Ott T, Krusell L, Voroshilova V, Ugalde RA, et al. Defects in rhizobial cyclic glucan and lipopolysaccharide synthesis alter legume gene expression during nodule development. Mol Plant Microbe Interact 2008;21:50–60. [PubMed: 18052882]
36. D'Haeze W, Glushka J, De Rycke R, Holsters M, Carlson RW. Structural characterization of extracellular polysaccharides of *Azorhizobium caulinodans* and importance for nodule initiation on *Sesbania rostrata*. Mol Microbiol 2004;52:485–500. [PubMed: 15066035]
37. D'Haeze W, Holsters M. Nod factor structures, responses, and perception during initiation of nodule development. Glycobiology 2002;12:79R–105R.
38. D'Haeze W, Leoff C, Freshour G, Noel KD, Carlson RW. *Rhizobium etli* CE3 bacteroid lipopolysaccharides are structurally similar but not identical to those produced by cultured CE3 bacteria. J Biol Chem 2007;282:17101–13. [PubMed: 17420254]
39. Davidson SK, Koropatnick TA, Kossmehl R, Sycuro L, McFall-Ngai MJ. NO means 'yes' in the squid-vibrio symbiosis: nitric oxide (NO) during the initial stages of a beneficial association. Cell Microbiol 2004;6:1139–51. [PubMed: 15527494]
40. Davies BW, Walker GC. Disruption of *sitA* compromises *Sinorhizobium meliloti* for manganese uptake required for protection against oxidative stress. J Bacteriol 2007;189:2101–9. [PubMed: 17172335]
41. Davies BW, Walker GC. Identification of novel *Sinorhizobium meliloti* mutants compromised for oxidative stress protection and symbiosis. J Bacteriol 2007;189:2110–13. [PubMed: 17172326]
42. de Cristobal RE, Vincent PA, Salomon RA. A combination of *sbmA* and *tolC* mutations in *Escherichia coli* K-12 Tn10-carrying strains results in hypersusceptibility to tetracycline. J Bacteriol 2008;190:1491–94. [PubMed: 18083810]
43. Deleted in proof
44. Denison RF, Kiers ET. Lifestyle alternatives for rhizobia: mutualism, parasitism, and forgoing symbiosis. FEMS Microbiol Lett 2004;237:187–93. [PubMed: 15321661]
45. Denison RF, Witty JF, Minchin FR. Reversible O_2 inhibition of nitrogenase activity in attached soybean nodules. Plant Physiol 1992;100:1863–68. [PubMed: 16653210]
46. Doherty D, Leigh JA, Glazebrook J, Walker GC. *Rhizobium meliloti* mutants that overproduce the *R. meliloti* acidic calcofluor-binding exopolysaccharide. J Bacteriol 1988;170:4249–56. [PubMed: 2842307]
47. Dunlap J, Minami E, Bhagwat AA, Keister DL, Stacey G. Nodule development induced by mutants of *Bradyrhizobium japonicum* defective in cyclic B-glucan synthesis. Mol Plant Microbe Interact 1996;9:546–55. [PubMed: 8810069]
48. Dylan T, Nagpal P, Helinski DR, Ditta GS. Symbiotic pseudorevertants of *Rhizobium meliloti ndv* mutants. J Bacteriol 1990;172:1409–17. [PubMed: 2307652]
49. El Yahyaoui F, Küster H, Ben Amor B, Hohnjec N, Pühler A, et al. Expression profiling in *Medicago truncatula* identifies more than 750 genes differentially expressed during nodulation, including many potential regulators of the symbiotic program. Plant Physiol 2004;136:3159–76. [PubMed: 15466239]
50. Endre G, Kereszt A, Kevei Z, Mihacea S, Kalo P, Kiss GB. A receptor kinase gene regulating symbiotic nodule development. Nature 2002;417:962–66. [PubMed: 12087406]
51. Esseling JJ, Lhuissier FG, Emons AM. Nod factor-induced root hair curling: continuous polar growth towards the point of nod factor application. Plant Physiol 2003;132:1982–88. [PubMed: 12913154]
52. Ferguson GP, Datta A, Baumgartner J, Roop RM 2nd, Carlson RW, Walker GC. Similarity to peroxisomal-membrane protein family reveals that *Sinorhizobium* and *Brucella* BacA affect lipid-A fatty acids. Proc Natl Acad Sci USA 2004;101:5012–17. [PubMed: 15044696]

53. Ferguson GP, Datta A, Carlson RW, Walker GC. Importance of unusually modified lipid A in *Sinorhizobium* stress resistance and legume symbiosis. Mol Microbiol 2005;56:68–80. [PubMed: 15773979]
54. Ferguson GP, Roop RM 2nd, Walker GC. Deficiency of a *Sinorhizobium meliloti* BacA mutant in alfalfa symbiosis correlates with alteration of the cell envelope. J Bacteriol 2002;184:5625–32. [PubMed: 12270820]
55. Fischer HM. Genetic regulation of nitrogen fixation in rhizobia. Microbiol Rev 1994;58:352–86. [PubMed: 7968919]
56. Foucher F, Kondorosi E. Cell cycle regulation in the course of nodule organogenesis in *Medicago*. Plant Mol Biol 2000;43:773–86. [PubMed: 11089876]
57. Fraysse N, Couderc F, Poinsot V. Surface polysaccharide involvement in establishing the rhizobium-legume symbiosis. Eur J Biochem 2003;270:1365–80. [PubMed: 12653992]
58. Fujishige NA, Lum MR, De Hoff PL, Whitelegge JP, Faull KF, Hirsch AM. Rhizobium common nod genes are required for biofilm formation. Mol Microbiol 2008;67:504–15. [PubMed: 18086203]
59. Gage DJ. Infection and invasion of roots by symbiotic, nitrogen-fixing rhizobia during nodulation of temperate legumes. Microbiol Mol Biol Rev 2004;68:280–300. [PubMed: 15187185]
60. Galibert F, Finan TM, Long SR, Puhler A, Abola P, et al. The composite genome of the legume symbiont *Sinorhizobium meliloti*. Science 2001;293:668–72. [PubMed: 11474104]
61. Gibson KE, Barnett MJ, Toman CJ, Long SR, Walker GC. The symbiosis regulator CbrA modulates a complex regulatory network affecting the flagellar apparatus and cell envelope proteins. J Bacteriol 2007;189:3591–602. [PubMed: 17237174]
62. Gibson KE, Campbell GR, Lloret J, Walker GC. CbrA is a stationary-phase regulator of cell surface physiology and legume symbiosis in *Sinorhizobium meliloti*. J Bacteriol 2006;188:4508–21. [PubMed: 16740957]
63. Giraud E, Moulin L, Vallenet D, Barbe V, Cytryn E, et al. Legumes symbioses: absence of Nod genes in photosynthetic bradyrhizobia. Science 2007;316:1307–12. [PubMed: 17540897]
64. Glazebrook J, Ichige A, Walker GC. A *Rhizobium meliloti* homolog of the *Escherichia coli* peptide-antibiotic transport protein SbmA is essential for bacteroid development. Genes Dev 1993;7:1485–97. [PubMed: 8393417]
65. Gleason C, Chaudhuri S, Yang T, Munoz A, Poovaiah BW, Oldroyd GE. Nodulation independent of rhizobia induced by a calcium-activated kinase lacking autoinhibition. Nature 2006;441:1149–52. [PubMed: 16810256]
66. Goedhart J, Hink MA, Visser AJ, Bisseling T, Gadella TW Jr. In vivo fluorescence correlation microscopy (FCM) reveals accumulation and immobilization of Nod factors in root hair cell walls. Plant J 2000;21:109–19. [PubMed: 10652156]
67. Gonzalez JE, Marketon MM. Quorum sensing in nitrogen-fixing rhizobia. Microbiol Mol Biol Rev 2003;67:574–92. [PubMed: 14665677]
68. Gruber N, Galloway JN. An Earth-system perspective of the global nitrogen cycle. Nature 2008;451:293–96. [PubMed: 18202647]
69. Deleted in proof
70. Hallez R, Bellefontaine AF, Letesson JJ, De Bolle X. Morphological and functional asymmetry in alpha-proteobacteria. Trends Microbiol 2004;12:361–65. [PubMed: 15276611]There are homologs of *C. crescentus* cell cycle regulators in a variety of symbiotic and pathogenic alphaproteobacteria,which also undergo asymmetric cell division.
71. Hallez R, Mignolet J, Van Mullem V, Wery M, Vandenhaute J, et al. The asymmetric distribution of the essential histidine kinase PdhS indicates a differentiation event in *Brucella abortus*. EMBO J 2007;26:1444–55. [PubMed: 17304218]
72. Harris JM, Wais R, Long SR. Rhizobium-lnduced calcium spiking in *Lotus japonicus*. Mol Plant Microbe Interact 2003;16:335–41. [PubMed: 12744462]
73. Harrison MJ. Signaling in the arbuscular mycorrhizal symbiosis. Annu Rev Microbiol 2005;59:19–42. [PubMed: 16153162]
74. Ichige A, Walker GC. Genetic analysis of the *Rhizobium meliloti bacA* gene: functional interchangeability with the *Escherichia coli sbmA* gene and phenotypes of mutants. J Bacteriol 1997;179:209–16. [PubMed: 8982000]

75. Imaizumi-Anraku H, Takeda N, Charpentier M, Perry J, Miwa H, et al. Plastid proteins crucial for symbiotic fungal and bacterial entry into plant roots. Nature 2005;433:527–31. [PubMed: 15616514]
76. Jamet A, Mandon K, Puppo A, Herouart D. H_2O_2 is required for optimal establishment of the *Medicago sativa*/*Sinorhizobium meliloti* symbiosis. J Bacteriol 2007;189:8741–45. [PubMed: 17921312]Host-derived ROS play a positive role in IT growth.
77. Jamet A, Sigaud S, Van de Sype G, Puppo A, Herouart D. Expression of the bacterial catalase genes during *Sinorhizobium meliloti-Medicago sativa* symbiosis and their crucial role during the infection process. Mol Plant Microbe Interact 2003;16:217–25. [PubMed: 12650453]
78. Jones KM, Kobayashi H, Davies BW, Taga ME, Walker GC. How rhizobial symbionts invade plants: the *Sinorhizobium-Medicago* model. Nat Rev Microbiol 2007;5:619–33. [PubMed: 17632573]
79. Jones KM, Sharopova N, Lohar DP, Zhang JQ, VandenBosch KA, Walker GC. Differential response of the plant *Medicago truncatula* to its symbiont *Sinorhizobium meliloti* or an exopolysaccharide-deficient mutant. Proc Natl Acad Sci USA 2008;105:704–9. [PubMed: 18184805]
80. Jordan A, Reichard P. Ribonucleotide Reductases. Annu Rev Biochem 1998;67:71–98. [PubMed: 9759483]
81. Jumas-Bilak E, Michaux-Charachon S, Bourg G, Ramuz M, Allardet-Servent A. Unconventional genomic organization in the alpha subgroup of the Proteobacteria. J Bacteriol 1998;180:2749–55. [PubMed: 9573163]
82. Kaneko T, Nakamura Y, Sato S, Asamizu E, Kato T, et al. Complete genome structure of the nitrogen-fixing symbiotic bacterium *Mesorhizobium loti*. DNA Res 2000;7:331–38. [PubMed: 11214968]
83. Kannenberg, E.; Reuhs, BL.; Forsberg, LS.; Carlson, RW. Lipopolysaccharides and K Antigens: Their Structures, Biosynthesis and Functions. Dordrecht, Neth; Kluwer: 1998. p. 119-54.
84. Kannenberg EL, Carlson RW. Lipid A and O-chain modifications cause *Rhizobium* lipopolysaccharides to become hydrophobic during bacteroid development. Mol Microbiol 2001;39:379–91. [PubMed: 11136459]
85. Kiers ET, Rousseau RA, West SA, Denison RF. Host sanctions and the legume-rhizobium mutualism. Nature 2003;425:78–81. [PubMed: 12955144]
86. Klein S, Walker GC, Signer ER. All *nod* genes of *Rhizobium meliloti* are involved in alfalfa nodulation by *exo* mutants. J Bacteriol 1988;170:1003–6. [PubMed: 3338972]
87. Kobayashi H, Naciri-Graven Y, Broughton WJ, Perret X. Flavonoids induce temporal shifts in gene-expression of nod-box controlled loci in *Rhizobium* sp NGR234. Mol Microbiol 2004;51:335–47. [PubMed: 14756776]
88. Kobayashi H, Simmons LA, Yuan DS, Broughton WJ, Walker GC. Multiple Ku orthologues mediate DNA nonhomologous end-joining in the free-living form and during chronic infection of *Sinorhizobium meliloti*. Mol Microbiol 2008;67:350–63. [PubMed: 18067541]
89. Kobayashi H, Sunako M, Hayashi M, Murooka Y. DNA synthesis and fragementation in bacteroids during *Astragalus sinicus* root nodule development. Biosci Biotechnol Biochem 2001;65:510–15. [PubMed: 11330661]
90. Kouchi H, Shimomura K, Hata S, Hirota A, Wu GJ, et al. Large-scale analysis of gene expression profiles during early stages of root nodule formation in a model legume, *Lotus japonicus*. DNA Res 2004;11:263–74. [PubMed: 15500251]
91. Kuppusamy KT, Endre G, Prabhu R, Penmetsa RV, Veereshlingam H, et al. *LIN*, a *Medicago truncatula* gene required for nodule differentiation and persistence of rhizobial infections. Plant Physiol 2004;136:3682–91. [PubMed: 15516512]
92. Kuzma MM, Hunt S, Layzell DB. Role of oxygen in the limitation and inhibition of nitrogenase activity and respiration rate in individual soybean nodules. Plant Physiol 1993;101:161–69. [PubMed: 12231675]
93. Lapaque N, Moriyon I, Moreno E, Gorvel JP. *Brucella* lipopolysaccharide acts as a virulence factor. Curr Opin Microbiol 2005;8:60–66. [PubMed: 15694858]
94. Lapaque N, Takeuchi O, Corrales F, Akira S, Moriyon I, et al. Differential inductions of TNF-alpha and IGTP, IIGP by structurally diverse classic and nonclassic lipopolysaccharides. Cell Microbiol 2006;8:401–13. [PubMed: 16469053]
95. Latch JN, Margolin W. Generation of buds, swellings, and branches instead of filaments after blocking the cell cycle of *Rhizobium meliloti*. J Bacteriol 1997;179:2373–81. [PubMed: 9079925]

96. Laviña M, Pugsley AP, Moreno F. Identification, mapping, cloning and characterization of a gene (*sbmA*) required for microcin B17 action on *Escherichia coli* K12. J Gen Microbiol 1986;6:1685–93.

97. Le Strange KK, Bender GL, Djordjevic MA, Rolfe BG, Redmond JW. The *Rhizobium* strain NGR234 *nodD1* gene product responds to activation by the simple phenolic compounds vanillin and isovanillian present in wheat seedling extracts. Mol Plant Microbe Interact 1990;3:214–20.

98. Leigh JA, Reed JW, Hanks JF, Hirsch AM, Walker GC. *Rhizobium meliloti* mutants that fail to succinylate their calcofluor-binding exopolysaccharide are defective in nodule invasion. Cell 1987;51:579–87. [PubMed: 2824062]

99. Leigh JA, Signer ER, Walker GC. Exopolysaccharide-deficient mutants of *Rhizobium meliloti* that form ineffective nodules. Proc Natl Acad Sci USA 1985;82:6231–35. [PubMed: 3862129]

100. LeVier K, Phillips RW, Grippe VK, Roop RM 2nd, Walker GC. Similar requirements of a plant symbiont and a mammalian pathogen for prolonged intracellular survival. Science 2000;287:2492–93. [PubMed: 10741969]A plant symbiont and a mammalian pathogen have similar genetic requirements for chronic host infection.

101. LeVier K, Walker GC. Genetic analysis of the *Sinorhizobium meliloti* BacA protein: differential effects of mutations on phenotypes. J Bacteriol 2001;183:6444–53. [PubMed: 11591690]

102. Li Y, Green LS, Holtzapffel R, Day DA, Bergersen FJ. Supply of O_2 regulates demand for O_2 and uptake of malate by N_2-fixing bacteroids from soybean nodules. Microbiology 2001;147:663–70. [PubMed: 11238973]

103. Limpens E, Franken C, Smit P, Willemse J, Bisseling T, Geurts R. LysM domain receptor kinases regulating rhizobial Nod factor-induced infection. Science 2003;302:630–33. [PubMed: 12947035]

104. Lohar DP, Haridas S, Gantt JS, VandenBosch KA. A transient decrease in reactive oxygen species in roots leads to root hair deformation in the legume-rhizobia symbiosis. New Phytol 2007;173:39–49. [PubMed: 17176392]

105. Lohar DP, Sharopova N, Endre G, Penuela S, Samac D, et al. Transcript analysis of early nodulation events in *Medicago truncatula*. Plant Physiol 2006;140:221–34. [PubMed: 16377745]

106. Lombardo F, Heckmann AB, Miwa H, Perry JA, Yano K, et al. Identification of symbiotically defective mutants of *Lotus japonicus* affected in infection thread growth. Mol Plant Microbe Interact 2006;19:1444–50. [PubMed: 17153928]

107. Luo L, Qi MS, Yao SY, Cheng HP, Zhu JB, Yu GQ. Role of *oxyR* from *Sinorhizobium meliloti* in regulating the expression of catalases. Acta Biochim Biophys Sin 2005;37:421–28. [PubMed: 15944758]

108. Ma X, Sun Q, Wang R, Singh G, Jonietz EL, Margolin W. Interactions between heterologous FtsA and FtsZ proteins at the FtsZ ring. J Bacteriol 1997;179:6788–97. [PubMed: 9352931]

109. Machado D, Krishnan HB. *nodD* alleles of *Sinorhizobium fredii* USDA191 differentially influence soybean nodulation, *nodC* expression, and production of exopolysaccharides. Curr Microbiol 2003;47:134–37. [PubMed: 14506861]

110. Margolin W, Corbo JC, Long SR. Cloning and characterization of a *Rhizobium meliloti* homolog of the *Escherichia coli* cell division gene *ftsZ*. J Bacteriol 1991;173:5822–30. [PubMed: 1653222]

111. Margolin W, Long SR. *Rhizobium meliloti* contains a novel second homolog of the cell division gene *ftsZ*. J Bacteriol 1994;176:2033–43. [PubMed: 8144471]

112. Marie C, Broughton WJ, Deakin WJ. Rhizobium type III secretion systems: legume charmers or alarmers? Curr Opin Plant Biol 2001;4:336–42. [PubMed: 11418344]

113. Mattiuzzo M, Bandiera A, Gennaro R, Benincasa M, Pacor S, et al. Role of the *Escherichia coli* SbmA in the antimicrobial activity of proline-rich peptides. Mol Microbiol 2007;66:151–63. [PubMed: 17725560]

114. Mergaert P, Uchiumi T, Alunni B, Evanno G, Cheron A, et al. Eukaryotic control on bacterial cell cycle and differentiation in the *Rhizobium*-legume symbiosis. Proc Natl Acad Sci USA 2006;103:5230–35. [PubMed: 16547129]Bacteroids undergo endoreduplication and are terminally differentiated cells.

115. Mitra RM, Gleason CA, Edwards A, Hadfield J, Downie JA, 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 2004;101:4701–5. [PubMed: 15070781]

116. Mitra RM, Shaw SL, Long SR. Six nonnodulating plant mutants defective for Nod factor-induced transcriptional changes associated with the legume-rhizobia symbiosis. Proc Natl Acad Sci USA 2004;101:10217–22. [PubMed: 15220482]

117. Moulin L, Munive A, Dreyfus B, Boivin-Masson C. Nodulation of legumes by members of the beta-subclass of Proteobacteria. Nature 2001;411:948–50. [PubMed: 11418858]

118. Nathan C, Shiloh MU. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. Proc Natl Acad Sci USA 2000;97:8841–48. [PubMed: 10922044]

119. Niehaus K, Kapp D, Puhler A. Plant defense and delayed infection of psuedonodules induced by an exopolysaccharide (EPS I)-deficient *Rhizobium meliloti* mutant. Planta 1993;190:415–25.

120. Ohwada T, Shirakawa Y, Kusumoto M, Masuda H, Sato T. Susceptibility to hydrogen peroxide and catalase activity of root nodule bacteria. Biosci Biotechnol Biochem 1999;63:457–62. [PubMed: 10227131]

121. Oke V, Long SR. Bacteroid formation in the Rhizobium-legume symbiosis. Curr Opin Microbiol 1999;2:641–46. [PubMed: 10607628]

122. Oldroyd GE, Downie JA. Coordinating nodule morphogenesis with rhizobial infection in legumes. Annu Rev Plant Biol 2008;59:519–46. [PubMed: 18444906]

123. Oldroyd GE, Mitra RM, Wais RJ, Long SR. Evidence for structurally specific negative feedback in the Nod factor signal transduction pathway. Plant J 2001;28:191–99. [PubMed: 11722762]

124. Ott T, van Dongen JT, Gunther C, Krusell L, Desbrosses G, et al. Symbiotic leghemoglobins are crucial for nitrogen fixation in legume root nodules but not for general plant growth and development. Curr Biol 2005;15:531–35. [PubMed: 15797021]

125. Ozga DA, Lara JC, Leigh JA. The regulation of exopolysaccharide production is important at two levels of nodule development in *Rhizobium meliloti*. Mol Plant Microbe Interact 1994;7:758–65. [PubMed: 7873780]

126. Parniske M, Schmidt PE, Kosch K, Muller P. Plant defense responses of host plants with determinate nodules induced by EPS-defective *exoB* mutants of *Bradyrhizobium japonicus*. Mol Plant Microbe Interact 1994;7:631–38.

127. Peck MC, Fisher RF, Long SR. Diverse flavonoids stimulate NodD1 binding to *nod* gene promoters in *Sinorhizobium meliloti*. J Bacteriol 2006;188:5417–27. [PubMed: 16855231]

128. Perret X, Staehelin C, Broughton WJ. Molecular basis of symbiotic promiscuity. Microbiol Mol Biol Rev 2000;64:180–201. [PubMed: 10704479]

129. Peters JW, Szilagyi RK. Exploring new frontiers of nitrogenase structure and mechanism. Curr Opin Chem Biol 2006;10:101–8. [PubMed: 16510305]

130. Philpott DJ, Girardin SE. The role of Toll-like receptors and Nod proteins in bacterial infection. Mol Immunol 2004;41:1099–108. [PubMed: 15476921]

131. Prell J, Poole P. Metabolic changes of rhizobia in legume nodules. Trends Microbiol 2006;14:161–68. [PubMed: 16520035]

132. Pueppke SG, Broughton WJ. *Rhizobium* sp strain NGR234 and *R fredii* USDA257 share exceptionally broad, nested host ranges. Mol Plant Microbe Interact 1999;12:293–318. [PubMed: 10188270]

133. Puppo A, Groten K, Bastian F, Carzaniga R, Soussi M, et al. Legume nodule senescence: roles for redox and hormone signalling in the orchestration of the natural aging process. New Phytol 2005;165:683–701. [PubMed: 15720680]

134. Radutoiu S, Madsen LH, Madsen EB, Jurkiewicz A, Fukai E, et al. LysM domains mediate lipochitin-oligosaccharide recognition and *Nfr* genes extend the symbiotic host range. EMBO J 2007;26:3923–35. [PubMed: 17690687]Just one amino acid change in a LysM domain can alter symbiosis host specificity.

135. Raetz CRH, Whitfield C. Lipopolysaccharide endotoxins. Annu Rev Biochem 2002;71:635–700. [PubMed: 12045108]

136. Ramu SK, Peng HM, Cook DR. Nod factor induction of reactive oxygen species production is correlated with expression of the early nodulin gene *rip1* in *Medicago truncatula*. Mol Plant Microbe Interact 2002;15:522–28. [PubMed: 12059100]

137. Raymond J, Siefert JL, Staples CR, Blankenship RE. The natural history of nitrogen fixation. Mol Biol Evol 2004;21:54154.

138. Reed JW, Glazebrook J, Walker GC. The *exoR* gene of *Rhizobium meliloti* affects RNA levels of other *exo* genes but lacks homology to known transcriptional regulators. J Bacteriol 1991;173:3789–94. [PubMed: 1711027]

139. Roche P, Debelle F, Maillet F, Lerouge P, Faucher C, et al. Molecular basis of symbiotic host specificity in *Rhizobium meliloti: nodH* and *nodPQ* genes encode the sulfation of lipo-oligosaccharide signals. Cell 1991;67:1131–43. [PubMed: 1760841]

140. Roop RM 2nd, Robertson GT, Ferguson GP, Milford LE, Winkler ME, Walker GC. Seeking a niche: putative contributions of the *hfq* and *bacA* gene products to the successful adaptation of the brucellae to their intracellular home. Vet Microbiol 2002;90:349–63. [PubMed: 12414155]

141. Rosendahl L, Vance CP, Pedersen WB. Products of dark CO_2 fixation in pea root nodules support bacteroid metabolism. Plant Physiol 1990;93:12–19. [PubMed: 16667422]

142. Rubio MC, James EK, Clemente MR, Bucciarelli B, Fedorova M, et al. Localization of superoxide dismutases and hydrogen peroxide in legume root nodules. Mol Plant Microbe Interact 2004;17:1294–305. [PubMed: 15597735]

143. Salminen SO, Streeter JG. Labeling of carbon pools in *Bradyrhizobium japonicum* and *Rhizobium leguminosarum* bv *viciae* bacteroids following incubation of intact nodules with CO_2. Plant Physiol 1992;100:597–604. [PubMed: 16653034]

144. Samac DA, Graham MA. Recent advances in legume-microbe interactions: recognition, defense response, and symbiosis from a genomic perspective. Plant Physiol 2007;144:582–87. [PubMed: 17556521]

145. Sanchez-Contreras M, Bauer WD, Gao M, Robinson JB, Allan Downie J. Quorum-sensing regulation in rhizobia and its role in symbiotic interactions with legumes. Philos Trans R Soc London Ser B 2007;362:1149–63. [PubMed: 17360278]

146. Santos R, Bocquet S, Puppo A, Touati D. Characterization of an atypical superoxide dismutase from *Sinorhizobium meliloti*. J Bacteriol 1999;181:4509–16. [PubMed: 10419947]

147. Santos R, Herouart D, Sigaud S, Touati D, Puppo A. Oxidative burst in alfalfa-*Sinorhizobium meliloti* symbiotic interaction. Mol Plant Microbe Interact 2001;14:86–89. [PubMed: 11194876]

148. Scheidle H, Gross A, Niehaus K. The lipid A substructure of the *Sinorhizobium meliloti* lipopolysaccharides is sufficient to suppress the oxidative burst in host plants. New Phytol 2005;165:559–65. [PubMed: 15720666]

149. Schultze M, Quiclet-Sire B, Kondorosi E, Virelizer H, Glushka JN, et al. *Rhizobium meliloti* produces a family of sulfated lipooligosaccharides exhibiting different degrees of plant host specificity. Proc Natl Acad Sci USA 1992;89:192–96. [PubMed: 1729688]

150. Sharypova LA, Niehaus K, Scheidle H, Holst O, Becker A. *Sinorhizobium meliloti acpXL* mutant lacks the C28 hydroxylated fatty acid moiety of lipid A and does not express a slow migrating form of lipopolysaccharide. J Biol Chem 2003;278:12946–54. [PubMed: 12566460]

151. Shaw SL, Long SR. Nod factor inhibition of reactive oxygen efflux in a host legume. Plant Physiol 2003;132:2196–204. [PubMed: 12913174]

152. Sigaud S, Becquet V, Frendo P, Puppo A, Herouart D. Differential regulation of two divergent *Sinorhizobium meliloti* genes for HPII-like catalases during free-living growth and protective role of both catalases during symbiosis. J Bacteriol 1999;181:2634–39. [PubMed: 10198032]

153. Simsek S, Ojanen-Reuhs T, Stephens SB, Reuhs BL. Strain-ecotype specificity in *Sinorhizobium meliloti–Medicago truncatula* symbiosis is correlated to succinoglycan oligosaccharide structure. J Bacteriol 2007;189:7733–40. [PubMed: 17766412]

154. Skerker JM, Laub MT. Cell-cycle progression and the generation of asymmetry in *Caulobacter crescentus*. Nat Rev Microbiol 2004;2:325–37. [PubMed: 15031731]

155. Skorupska A, Janczarek M, Marczak M, Mazur A, Krol J. Rhizobial exopolysaccharides: genetic control and symbiotic functions. Microb Cell Fact 2006;5:7. [PubMed: 16483356]

156. Soto MJ, Sanjuan J, Olivares J. Rhizobia and plant-pathogenic bacteria: common infection weapons. Microbiology 2006;152:3167–74. [PubMed: 17074888]

157. Spaink HP. Root nodulation and infection factors produced by rhizobial bacteria. Annu Rev Microbiol 2000;54:257–88. [PubMed: 11018130]

158. Sprent JI. 60 Ma of legume nodulation. What's new? What's changing? J Exp Bot 2008;59:1081–84. [PubMed: 18209109]

159. Stracke S, Kistner C, Yoshida S, Mulder L, Sato S, et al. A plant receptor-like kinase required for both bacterial and fungal symbiosis. Nature 2002;417:959–62. [PubMed: 12087405]

160. Sullivan JT, Patrick HN, Lowther WL, Scott DB, Ronson CW. Nodulating strains of *Rhizobium loti* arise through chromosomal symbiotic gene transfer in the environment. Proc Natl Acad Sci USA 1995;92:8985–89. [PubMed: 7568057]

161. Sullivan JT, Ronson CW. Evolution of rhizobia by acquisition of a 500-kb symbiosis island that integrates into a phe-tRNA gene. Proc Natl Acad Sci USA 1998;95:5145–49. [PubMed: 9560243]

162. Taga ME, Larsen NA, Howard-Jones AR, Walsh CT, Walker GC. BluB cannibalizes flavin to form the lower ligand of vitamin B_{12}. Nature 2007;446:449–53. [PubMed: 17377583]

163. Teillet A, Garcia J, de Billy F, Gherardi M, Huguet T, et al. *api*, A novel *Medicago truncatula* symbiotic mutant impaired in nodule primordium invasion. Mol Plant Microbe Interact 2008;21:535–46. [PubMed: 18393613]

164. Tellstrom V, Usadel B, Thimm O, Stitt M, Kuster H, Niehaus K. The lipopolysaccharide of *Sinorhizobium meliloti* suppresses defense-associated gene expression in cell cultures of the host plant *Medicago truncatula*. Plant Physiol 2007;143:825–37. [PubMed: 17220366]

165. Theunis M, Kobayashi H, Broughton WJ, Prinsen E. Flavonoids, NodD1, NodD2, and nod-box NB15 modulate expression of the y4wEFG locus that is required for indole-3-acetic acid synthesis in *Rhizobium* sp strain NGR234. Mol Plant Microbe Interact 2004;17:1153–61. [PubMed: 15497408]

166. Tirichine L, Imaizumi-Anraku H, Yoshida S, Murakami Y, Madsen LH, et al. Deregulation of a Ca^{2+}/calmodulin-dependent kinase leads to spontaneous nodule development. Nature 2006;441:1153–56. [PubMed: 16810257]

167. Tjepkema JD, Yocum CS. Measurement of oxygen partial pressure within soybean nodules by oxygen microelectrodes. Planta 1984;119:351–60.

168. Turner SL, Young JP. The glutamine synthetases of rhizobia: phylogenetics and evolutionary implications. Mol Biol Evol 2000;17:309–19. [PubMed: 10677854]

169. Urzainqui A, Walker GC. Exogenous suppression of the symbiotic deficiencies of *Rhizobium meliloti exo* mutants. J Bacteriol 1992;174:34036.

170. Vance CP. Symbiotic nitrogen fixation and phosphorus acquisition: plant nutrition in a world of declining renewable resources. Plant Physiol 2001;127:390–97. [PubMed: 11598215]

171. Vasse J, Billy F, Truchet G. Abortion of infection during the *Rhizobium meliloti*-alfalfa symbiotic interaction is accompanied by a hypersensitive reaction. Plant J 1993;4:555–66.

172. Vasse J, de Billy F, Camut S, Truchet G. Correlation between ultrastructural differentiation of bacteroids and nitrogen fixation in alfalfa nodules. J Bacteriol 1990;172:4295–306. [PubMed: 2376562]

173. Vedam V, Haynes JG, Kannenberg EL, Carlson RW, Sherrier DJ. A *Rhizobium leguminosarum* lipopolysaccharide lipid-A mutant induces nitrogen-fixing nodules with delayed and defective bacteroid formation. Mol Plant Microbe Interact 2004;17:283–91. [PubMed: 15000395]

174. Vedam V, Kannenberg E, Datta A, Brown D, Haynes-Gann JG, et al. The pea nodule environment restores the ability of a *Rhizobium leguminosarum* lipopolysaccharide *acpXL* mutant to add 27-hydroxyoctacosanoic acid to its lipid A. J Bacteriol 2006;188:2126–33. [PubMed: 16513742]

175. Vedam V, Kannenberg EL, Haynes JG, Sherrier DJ, Datta A, Carlson RW. A *Rhizobium leguminosarum* AcpXL mutant produces lipopolysaccharide lacking 27-hydroxyoctacosanoic acid. J Bacteriol 2003;185:1841–50. [PubMed: 12618448]

176. Veereshlingam H, Haynes JG, Penmetsa RV, Cook DR, Sherrier DJ, Dickstein R. *nip*, a symbiotic *Medicago truncatula* mutant that forms root nodules with aberrant infection threads and plant defense-like response. Plant Physiol 2004;136:3692–702. [PubMed: 15516506]

177. Wais RJ, Galera C, Oldroyd G, Catoira R, Penmetsa RV, et al. Genetic analysis of calcium spiking responses in nodulation mutants of *Medicago truncatula*. Proc Natl Acad Sci USA 2000;97:13407–12. [PubMed: 11078514]

178. Wais RJ, Keating DH, Long SR. Structure-function analysis of nod factor-induced root hair calcium spiking in Rhizobium-legume symbiosis. Plant Physiol 2002;129:211–24. [PubMed: 12011352]

179. Walker SA, Viprey V, Downie JA. Dissection of nodulation signaling using pea mutants defective for calcium spiking induced by nod factors and chitin oligomers. Proc Natl Acad Sci USA 2000;97:13413–18. [PubMed: 11078515]

180. Wan J, Zhang XC, Neece D, Ramonell KM, Clough S, et al. A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in *Arabidopsis*. Plant Cell 2008;20:471–81. [PubMed: 18263776]

181. Wanders RJ, Visser WF, van Roermund CW, Kemp S, Waterham HR. The peroxisomal ABC transporter family. Pflugers Arch 2007;453:719–34. [PubMed: 17039367]

182. Wang LX, Wang Y, Pellock B, Walker GC. Structural characterization of the symbiotically important low-molecular-weight succinoglycan of *Sinorhizobium meliloti*. J Bacteriol 1999;181:6788–96. [PubMed: 10542182]

183. Wells DH, Chen EJ, Fisher RF, Long SR. ExoR is genetically coupled to the ExoS-ChvI two-component system and located in the periplasm of *Sinorhizobium meliloti*. Mol Microbiol 2007;64:647–64. [PubMed: 17462014]

184. West SA, Kiers ET, Simms EL, Denison RF. Sanctions and mutualism stability: Why do rhizobia fix nitrogen? Proc Biol Sci 2002;269:685–94. [PubMed: 11934359]

185. White J, Prell J, James EK, Poole P. Nutrient sharing between symbionts. Plant Physiol 2007;144:604–14. [PubMed: 17556524]

186. Witty JF, Skot L, Revsbech NP. Direct evidence for changes in the resistance of legume root nodules to O_2 diffusion. J Exp Bot 1987;38:1129–40.

187. Wojtaszek P. Oxidative burst: an early plant response to pathogen infection. Biochem J 1997;322 (Part 3):681–92. [PubMed: 9148737]

188. Wong K, Golding GB. A phylogenetic analysis of the pSymB replicon from the *Sinorhizobium meliloti* genome reveals a complex evolutionary history. Can J Microbiol 2003;49:269–80. [PubMed: 12897836]

189. Yano K, Tansengco ML, Hio T, Higashi K, Murooka Y, et al. New nodulation mutants responsible for infection thread development in *Lotus japonicus*. Mol Plant Microbe Interact 2006;19:801–10. [PubMed: 16838792]

190. Zasloff M. Reconstructing one of nature's designs. Trends Pharmacol Sci 2000;21:236–38. [PubMed: 10871888]

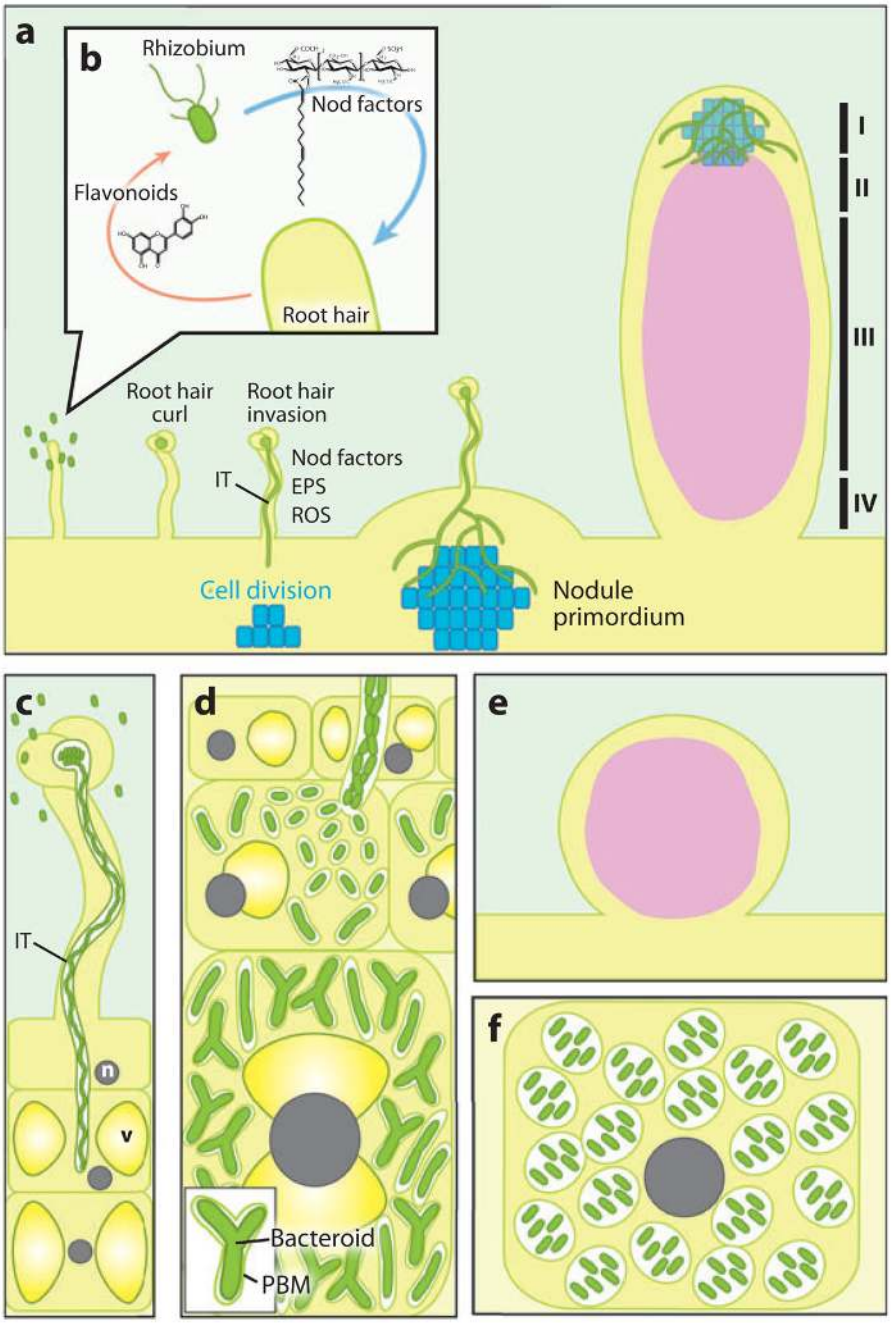

Figure 1.

Schematic model of nodule development. (*a–b*) Host flavonoids exuded into the soil trigger bacterial Nod Factor production. Nod factor is perceived by host receptors and elicits various host responses, such as root hair curling and root hair invasion. Root hair invasion also requires bacteria EPS and host ROS production. Nod factors induce mitotic cell division in the root cortex (represented in *blue*), leading to formation of the nodule meristem. An indeterminate nodule originates from the root inner cortex and has a persistent meristem (Zone I). The nodule also contains an invasion zone (Zone II) and a nitrogen-fixing zone (Zone III). In older nodules, a senescent zone (Zone IV) develops in which both plant and bacterial cells degenerate. (*c*) Bacteria enter the nodule through root hairs in a structure called the infection thread (IT) that

elongates toward the nodule meristem; nucleus (n), vacuole (v). (*d*) At the tip of the growing IT, bacteria are endocytosed into the cytoplasm of postmitotic endoploid cells. Each bacterium is surrounded by a host-derived peribacteroid membrane (PBM) and proceeds to differentiate into the specialized symbiotic form called a bacteroid. Bacteroids establish a chronic infection of the host cytoplasm and enzymatically reduce dinitrogen to provide a source of biologically usable nitrogen to the host (Zone III). (*e*) In contrast to an indeterminate nodule, a determinate nodule lacks a persistent meristem and all developmental stages proceed synchronously. (*f*) Infected cells of determinate nodules typically lack vacuoles (v).

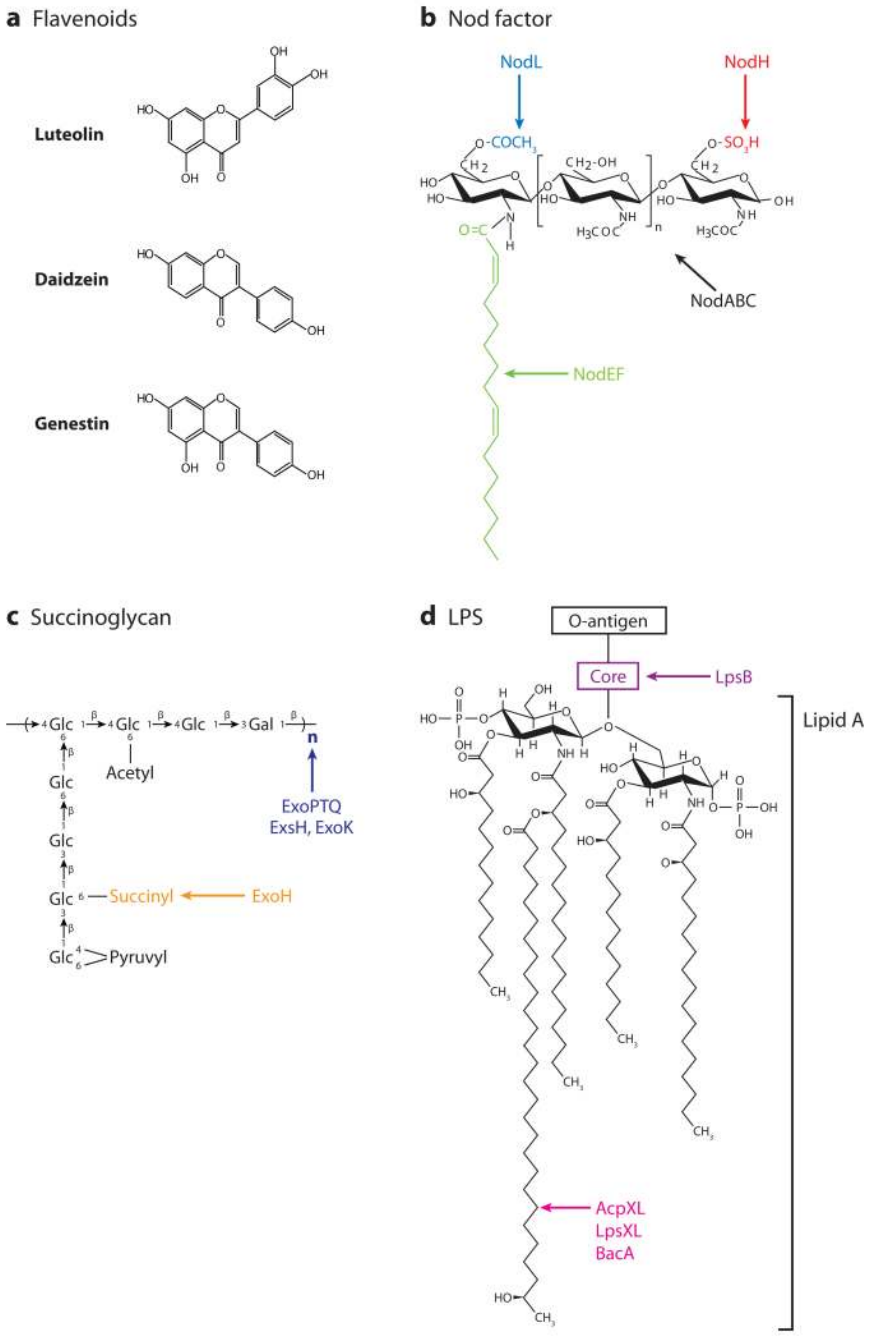

Figure 2.

Representative signaling molecules critical for symbiosis. (*a*) Host flavonoids: luteolin, daidzein, and genestin. (*b*) The Nod factor produced by *S. meliloti* and biosynthetic enzymes (Nod proteins) involved in its synthesis. (*c*) The *S. meliloti* exopolysaccharide succinoglycan. ExoH is responsible for succinyl modification; succinoglycan molecular weight is controlled by ExoPTQ and two extracellular glycosylases, ExsH and ExoK. (*d*) Schematic representation of *S. meliloti* lipopolysaccharide (LPS). LpsB is a glycosyltransferase with broad substrate specificity involved in synthesis of the LPS core. AcpXL, LpsXL, and BacA are required for the Very-Long-Chain Fatty Acid (C28) modification of lipid A.

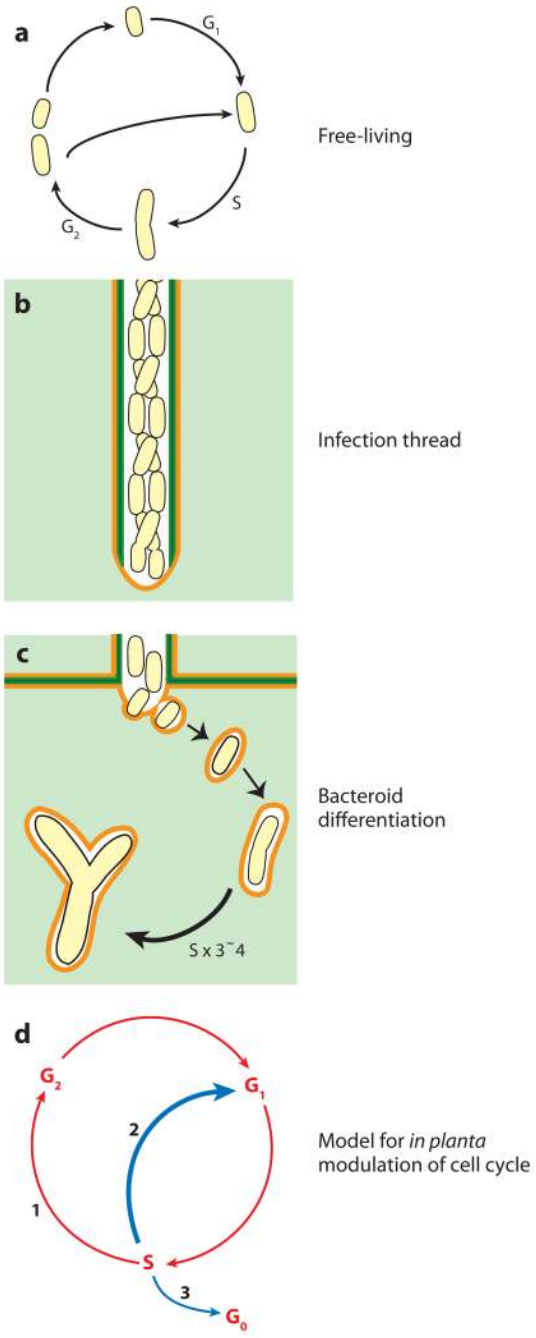

Figure 3.

Schematic representation of the rhizobium cell cycle at different stages of symbiosis. (*a*) The *S. meliloti* cell cycle is modeled after that of the alphaproteobacterium *Caulobacter crescentus*. A cell division cycle is comprised of three distinct phases: G_1, S, and G_2. In *C. crescentus*, DNA replication is initiated only once per cell cycle, in S phase; however, chromosome segregation begins during S phase and continues in G_2 phase. Cell division begins in G_2 phase and is completed before the next DNA replication initiation event. During free-living growth, *S. meliloti* is thought to initiate DNA replication only once per cell cycle and divides asymmetrically to produce daughter cells of different size. In analogy to *C. crescentus*, the small daughter cell likely proceeds into G_1 phase while the larger daughter cell

directly re-enters S phase. (*b*) *S. meliloti* proliferating in the IT originate from a clonal expansion of founder cells entrapped in the tip of the root hair curl. Cells appear to lack flagella and are loosely associated with one another in a pole-to-pole manner, typically forming two or three columns with a braided appearance. Active propagation of bacteria is observed only in a limited area called the growth zone near the tip of the IT, while bacteria outside of the growth zone do not grow or divide. It seems likely that the restricted growth of bacteria enables synchronization of bacterial growth with extension of the IT. (*c*) Bacteria colonize the cytoplasm of plant cells located in the invasion zone (see Figure 1*d*). Bacteria are surrounded by a plant-derived membrane and differentiate into a bacteroid. In *S. meliloti*, DNA endoreduplication occurs during bacteroid differentiation and results in dramatic cell branching and enlargement to a length of 5–10 μm; in comparison, free-living counterparts are rod-shaped cells of 1–2 μm. These terminally differentiated (G_0 phase) bacteroids are unable to resume future growth. Orange lines, host plasma membrane; green lines, host cell wall. (*d*) A model of the *S. meliloti* cell cycle in planta has three possible exits from S phase, two of which (*in blue*) represent an exit from the typical free-living cycle (*in red*). Bacteria within the infection thread are thought to progress through the cell cycle in the same manner as free-living cells, and in particular transition from S phase into G_2 phase (represented by arrow 1). Bacteria that undergo bacteroid differentiation undertake the process of endoreduplication and therefore re-enter G_1 phase after the completion of S phase (represented by arrow 2); the bacteria may cycle from S to G_1 multiple times during endoreduplication. Once endoreduplication is complete, the bacteroid enters a terminally differentiated state (G_0) and is no longer able to initiate cellular growth or DNA replication (represented by arrow 3).

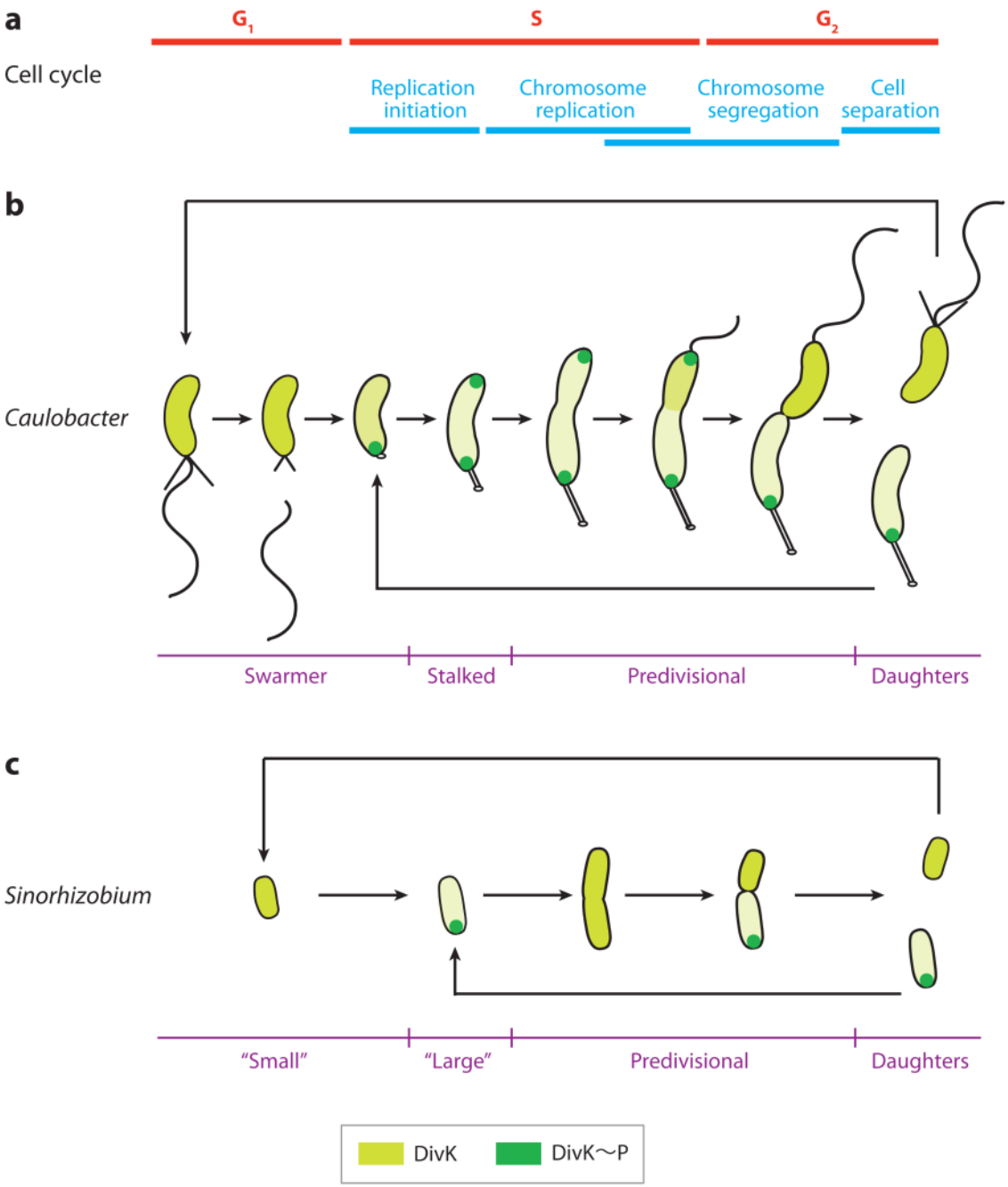

Figure 4.

Schematic representation of cell-cycle progression in *C. crescentus* and *S. meliloti*. (*a*) The cell cycle is comprised of three distinct phases: G_1, S, and G_2 (shown in *red*). The timing of cell cycle-related events is shown in blue. (*b*) During G_1 phase, *C. crescentus* is a swarmer cell, which is motile and has a polar flagellum and pili. When entering S phase, the swarmer cell ejects the flagellum, retracts the pili, and differentiates into a stalked cell. The stalked cell is uniquely competent to initiate DNA replication. After chromosome segregation in G_2 phase, the cell divides asymmetrically to produce two different daughter cells: a swarmer and a stalked cell. During cell-cycle progression, cellular localization of DivK is controlled by phosphorylation: nonphosphorylated DivK is uniformly distributed in the cytoplasm and

DivK~P is localized to the cell poles. (*c*) Recently it was shown that *S. meliloti* also divides asymmetrically to form a “small” cell and a “large” cell. Localization of the DivK homolog indicates that the “small” and “large” cells are counterparts of the *C. crescentus* swarmer and stalked cells, respectively. Flagella are omitted from this scheme because their localization has not been examined during cell cycle progression in *S. meliloti*.