Role of lectins (and rhizobial exopolysaccharides) in legume nodulation  
Ann M Hirsch

The lectin recognition hypothesis proposes that plant lectins mediate specificity in the *Rhizobium*-legume symbiosis. Although the hypothesis was developed eight years before *nod* genes were identified in rhizobia and sixteen years before Nod factor was shown to be a major determinant of host specificity, experiments performed recently using transgenic lectin plants support its main tenets.

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Abbreviations  
Ccd  cortical cell divisions  
CPS  capsular polysaccharides  
EPS  exopolysaccharides  
Had  root hair deformation  
LPS  lipopolysaccharides  
PRA  peanut root lectin  
PSL  *Pisum sativum* lectin  
SBL  *soybean lectin*

Introduction
The lectin-recognition hypothesis, championed by Hamblin and Kent [1], Bohlool and Schmidt [2], and Dazzo and Hubbell [3], was formulated to explain host specificity between legume and rhizobial symbiotic partners. It was based on the strong correlation between the inoculation specificity of bacteria of the family *Rhizobiaceae* on their legume hosts and the ability of host-produced lectins to bind to *Rhizobium* cells, and soon became accepted as ‘dogma’. The concept and the diagrammatic scheme shown in Figure 1 were incorporated into numerous textbooks, and although there were exceptions to the idea that plant lectins mediated specificity, the hypothesis has held for more than 20 years.

In this review, I will give a brief overview of the lectin recognition hypothesis, the experiments that confirm it, and offer some suggestions as to future research directions.

Why lectins?
Lectins were originally defined as carbohydrate-binding proteins of non-immune origin that agglutinate cells and/or precipitate glycoconjugates [4]. This definition has been modified for plant lectins to include those proteins possessing at least one noncatalytic domain that binds reversibly to a specific mono- or oligosaccharide [5]. Most plant lectins are multivalent. Some also bind small hydrophobic molecules such as adenine and derivatives, the cytokinins, which function as plant growth regulators [6,7].

Lectins correlate with the cross-inoculation groups established by their source legume [8]. Rhizobia that nodulate *Phaseolus vulgaris* were found to interact with bean lectin [1], whereas soybean lectin bound and aggregated 22 of 25 strains of *Bradyrhizobium* (previously known as *Rhizobium japonicum*) that nodulate soybean [2]. Twenty-three other bradyrhizobial strains that were not aggregated by SBL (soybean lectin) did not initiate nod-ule formation on soybean.

The lectin recognition hypothesis was formulated many years before the isolation and characterization of *nod* genes in rhizobia as well as before Nod factor was recognized as mediating legume-*Rhizobium* specificity. Generic Nod factor is an *N*-acetyl-glucosamine oligomer (with a backbone of three to five glucosamine units) and an acyl tail of differing lengths and saturation, depending on the rhizobial species. Various other substituents can be present on the reducing end [9] (see WJ Broughton and X Perret, this issue pp 305–311). Specificity resides in both the reducing and non-reducing ends of the molecule. On alfalfa, Nod factor alone, at a very low concentration (10^-12 M), elicits root hair deformation (Had), whereas a higher concentration (10^-7 M) is required for extensive cortical cell divisions (Ccd) [10]. Although many legumes undergo the Had
Lectins are found in the right place and at the right time

Most plant lectins are secreted proteins and are found in vacuoles, cell walls, or intercellular spaces. Although they are found in just about every plant organ, their distribution in root tissue gives the most support to the lectin recognition hypothesis — lectins are localized to root hairs, which are the sites of rhizobial entry for many legumes [15–20]. They are most frequently found at the tips of developing root hairs, although Díaz et al. [18] localized PSL to root hair precursor cells.

Root and seed lectins can be the product of a single gene. This is the case for soybean (Glycine max) [21] and its lectin SBL (or SBA) and pea (Pisum sativum) and its lectin PSL (or PSA) [22]. DNA gel blot hybridization and S1 nuclease analysis and ribonuclease protection experiments (used for fine-structure mapping and accurately determining the steady-state level of any mRNA) failed to identify any functional lectin genes in soybean other than Le1, the gene encoding SBL [21]. Although several papers suggested that there is a separate soybean root lectin, we were unable to find one. Our attempts to isolate a root protein that was reported to cross-react to an antibody made to SBL failed. Although we used the same antibody as Vodkin and Raikhel [23], the protein that we isolated had a high degree of sequence similarity to the acidic ribosomal protein P0 [24]. Thus, if there is a root lectin in soybean, it must be quite different from the protein encoded by Le1. The presence of another lectin may also explain why ‘Sooty’, a soybean cultivar with a mutant lectin gene (le1) nodulates [25], and why root exudate from Sooty reverses the delayed nodulation phenotype of B. japonicum strain HS111 in the same way as the lectin derived from Le1 soybeans [26]. Another lectin composed of 45 kDa subunits with specificity for 4-O-methylglucuronic acid [27,28] has been detected in soybean, but the gene encoding it remains unidentified. However, ‘Sooty’ has been tested only with high concentrations of inoculum (S Pueppke, personal communication). Will it nodulate with a below-threshold number of bradyrhizobia or with bradyrhizobia that do not attach properly (see later section)?

What bacterial component binds to legume lectins?

Rhizobia are typical Gram-negative bacteria with a cytoplasmic and outer membrane enclosing a periplasmic space. Much of their outer surface is composed of polysaccharides: lipopolysaccharides (LPS), capsular polysaccharides (CPS or KPS), and acidic exopolysaccharides (EPS). Mutants that are defective in the production of these polysaccharides are often blocked in inducing nodule development, particularly in steps involving nodule invasion via infection threads. A considerable amount of data show that rhizobial surface polysaccharides bind to some lectins.

An alfalfa lectin isolated from seed extract by Kamberger [31] was found in agarose gel diffusion tests to interact with Rhizobium (Sinorhizobium) meliloti LPS, but not with LPS from heterologous rhizobia. Jayaraman and Das [32] reported that the peanut root lectin (PRA II) bound to LPS of peanut-specific bradyrhizobia, and not to other Bradyrhizobium species.

New life for the lectin recognition hypothesis

The experiments of Díaz et al. [33] revitalized interest in the lectin recognition hypothesis. Transgenic white clover roots carrying the pea lectin gene were nodulated by the heterologous strain R. leguminosarum bv. viciae, whereas no nodules formed on clover plants that lacked the pea gene. Altering the carbohydrate-binding domain of the introduced lectin by site-directed mutagenesis demonstrated that lectin mediated this heterologous interaction. When transgenic plants carrying the mutated, transgenic lectin were inoculated with the heterologous rhizobia, no nodules formed [34].

We [13••] expanded the applicability of these experiments by investigating soybean and Lotus corniculatus [35], two legumes that are distantly related. We transferred Le1 into L. corniculatus, which is normally nodulated by...
Experiments on the transgenic *L. coroniculatus* showed the following: first, SBL was expressed in the emerging root hairs in the susceptible zone of the root; second, when the transgenic *Lotus* roots were inoculated with *B. japonicum*, nodules formed, probably because of the high concentration of heterologous Nod factor brought about by the accumulation of bradyrhizobia at the root hair tips. However, the nodules were devoid of bacteria because infection threads aborted; third, if the sugar-binding site of *Le1* was mutated and transgenic plants carrying the mutated lectin gene were inoculated with *B. japonicum*, no nodules developed even though the defective lectin was properly targeted to the root hair tip. The bradyrhizobia did not attach to these root hairs [13••], and, presumably, a high concentration of heterologous Nod factor was no longer present at the root hair tip. These experiments indicated that lectins mediate one level of host specificity, and furthermore, that some component of the bradyrhizobial cell surface must bind to SBL. Although *B. japonicum* USDA110 induced nodules on the roots of the transgenic *Lotus*, neither *B. elkanii* USDA31, which does not bind SBL, nor *B. japonicum* exoB mutants, attached to the *Lotus* roots or induced nodules over the levels demonstrated by the control plants [13••]. These results suggested that mediation of host specificity occurs via lectin interacting with an exopolysaccharide component of the rhizobial cell surface.

If exopolysaccharide is a ligand for lectin, one would predict that Exo− rhizobia would be unable to attach to plant root hairs or to elicit infection thread formation. The former is not true, most likely because other rhizobial proteins also function in adhesion (see references in [36]). However, the latter is frequently true. Inoculation with rhizobial exopolysaccharide mutants phenocopies the nodules developed by the transgenic lectin plants. Uninfected nodules develop in response to Exo− mutants because infection threads abort [37,38••]. For *Sinorhizobium meliloti* strain 1021, succinoglycan appears to be essential for colonization of the root hair. Mutants that either do not produce or overproduce succinoglycan fail to form proper infection threads [38••]. Exo− mutants of *R. leguminosarum* bv. *trifolii* also elicit uninfected nodules [39,40•].

How is lectin mediating specificity in the transgenic plants? One possibility is that lectins are a ‘glue’ that attaches the rhizobia to a site on a susceptible root hair, in this way providing a point source of rhizobial-produced signal molecules. First, secreted lectin aggregates the rhizobia and facilitates their attachment to the root hair (Figure 2). Next, the bacteria elicit infection thread formation even though they produce a heterologous Nod factor. Nod− rhizobia do not induce infection threads or induce Ccd on the transgenic roots, arguing for the involvement of Nod factor in these processes [13••]. That infection threads are formed in the root hairs of the transgenic plants suggests that lectins mediate rhizobial attachment even within the thread (Figure 2). Nevertheless, even though the lectin and the surface polysaccharides are complementary, the threads abort and the nodules are uninfected, suggesting that there is some required response that is not duplicated in the transgenic plants. One possibility is that the transgenic lectin may not be properly integrated into a nodulation signal transduction pathway.

**Plant receptors for lectins?**

Lectins bind glycoconjugates found on the surfaces of foreign organisms. Indeed, lectins may serve as a sort of ‘cloaking’ device thereby facilitating the entry of a pathogenic microorganism. Brelles-Marino et al. [41] found that infection thread development was enhanced when *R. etli* was incubated with bean seed lectin. Does lectin mask rhizobial cell surface components such that the root hair cell takes up these foreign cells?

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A larger question is how the bacteria are engulfed once they make contact with the root hair. The lectins used to formulate the lectin recognition hypothesis have no transmembrane domains that could connect directly to components of a signal transduction cascade. Legume seed lectins are usually hololectins with two or more carbohydrate-binding sites, one per subunit; the carbohydrate binding domains are identical or very similar [42]. Proteins that bind to legume lectins (potential lectin receptors) have been detected in seeds (see references in [8]), but minimal information exists on their subcellular location [43]. The significance of these protein associations is also largely unknown.

It is possible that lectins function in signal transduction pathways by binding to transmembrane proteins. Metcalf et al. [44] found that soybean protoplasts bound several iodinated lectins specifically and saturably. Fluorescence microscopy of the protoplasts showed uniform labeling of the plasma membrane implying that the lectins bound to transmembrane receptors. The lectin–receptor complexes were mobile, as demonstrated by photobleaching studies, and mild trypsinization of the soybean protoplasts removed SBL, but not other lectins [44]. These findings demonstrate that receptors specific for SBL are localized to the plasma membrane.

A gene encoding a new class of receptor-like kinase has been cloned from Arabidopsis [45]. It appears to be a member of a superfamily of genes that define a new class of plant serine/threonine receptor kinases (see Note added in proof). What makes this putative receptor kinase unusual is that, on the basis of sequence information, the extracellular domain is homologous to a legume lectin, and has binding sites for sugars and for small hydrophobic molecules ([45]; see Note added in proof). However, there are also some significant differences from legume lectins based on molecular modelling, such as the lack of a Ca\textsuperscript{2+} binding site, which may be coupled to a lack of monosaccharide binding activity (see Note added in proof).

Are there proteins in legumes similar to the Arabidopsis lectin–kinase, or do legume lectins exist as soluble proteins that interact with transmembrane kinases once a ligand is bound? Ridge et al. [46••] tested the binding of several lectins with affinities for different sugars on root hairs of uninoculated legumes. They found that five of ten tested lectins bound to the broad-host range legume siratro (Macroptilium), whereas only one lectin bound to each of the narrow-host range plants, except for white clover, which bound two. No lectins bound to Arabidopsis root hairs. Sugars at the tips of the root hairs probably exist as glycoconjugates that are transiently expressed. What are these sugar-binding molecules at the root hair tip and are they involved in rhizobial entry? Could they be part of the signal transduction chain?
Conclusions and predictions

As it stands, the lectin recognition hypothesis — with a few modifications — has withstood the test of time. The question remains, however, as to whether lectin is merely a glue that holds the rhizobia and the plant in close contact until Nod factor works its wonders, or whether it plays an active role in specificity by transmitting a signal to one or both symbiotic partners. For example, Kijne and co-workers [47] proposed that lectins may be involved in stabilizing the cell cytoskeleton by transmembrane interactions. When lectin binds rhizobia, the root hair tip becomes destabilized and an infection thread can form.

Figure 3 illustrates other possibilities. In an uninoculated root hair (Figure 3a), Nod factor receptors and lectins are randomly distributed in and along the membrane of the root hair tip. Adding Nod factor by itself does not cause receptors/binding proteins to migrate even though Nod factor is bound to them; thus, the root hair deforms but does not curl (Figure 3b). When rhizobia are added, the root hair curls around the attached bacteria (Figure 3c). Attachment facilitated by lectins binding to their polysaccharide coats concentrates the rhizobia at a spot on the root hair. Nod factor synthesized by the rhizobia then binds to specific receptor/binding proteins that migrate laterally and form a focused cap (Figure 3d). The size of the cap dictates the strength of the ensuing signal transduction pathway and whether or not the rhizobia will be taken up into the infection thread [48].

In another model, lectins complex with transmembrane proteins, which, in the uninoculated root, are distributed randomly at the root-hair tip (Figure 3e). Upon inoculation, the lectin binds to the rhizobial surface and then interacts via a protein-binding domain with the putative transmembrane protein. The complexes cap and, in the process, the transmembrane proteins transmit the signal through an as yet uncharacterized nodulation signal transduction pathway (Figure 3f). Nod factor is still required for rhizobial entry because even though nodules develop, bona fide infection threads do not form without rhizobia contacting the root hair cell membrane. However, the effect of Nod factor may be independent of a receptor or binding protein. It is known that Nod factor can modify the biological activity of the root hair cell even in the absence of rhizobia (see references in [9]). Whether or not it does this through an external Nod Factor receptor/binding protein as indicated in Figure 3b or via another mechanism is unknown.

One test of the above possibilities is to determine whether cv. ‘Sooy’ nodulates in response to a low concentration of wild-type bradyrhizobia or to exoB B. japonicum. If there is too little Nod factor brought about by the reduced binding of bradyrhizobia to Sooy root hairs, then the roots will not form nodules.

Many of the components needed for rhizobial endocytosis have been found in legume root hair tips. In addition to lectin, numerous features that are involved in mediating bacterial invasion into host cells — actin, microtubules, clathrin, and various vesicles — have been detected [49,50]. However, we are still missing the critical components of the signal transduction pathway, although attempts to find them are underway. Then, it may be possible to test whether lectins play an active role in propagating a signal that leads to nodule development.

Note added in proof

Two papers have recently been published that are highly relevant to this review [52•,53•]. See main text for details.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest


Confirmed the validity of the lectin recognition hypothesis, and expanded upon the findings of [33] by showing that the lectin recognition hypothesis is applicable to legumes and rhizobia that are not closely related to each other. It also demonstrated that exopolysaccharide is a likely ligand for soybean lectin.


This combination of molecular and biochemical approaches demonstrates that a novel lectin, named LNP, is a nod factor-binding protein as well as an apyrase. LNP has no similarity in sequence to any lectins that have been described so far. Its putative location on a membrane suggests that it may function in oligosaccharide-signalling events.


19. Slodki ME: Green fluorescent protein (GFP) was used as reporter for the purpose of tracking the degree of infection thread formation. Infection threads elicited by clover rhizobia mutants penetrate nodule cells, but similar to the meliloti mutants, endocytosis is defective.


A promising approach showing that lectins may be binding to glycoconjugates, which could function in the nodulation signal transduction pathway. These glycoconjugates are likely to be an integral part of the plasma membrane at the root hair tip and may represent specific receptors for rhizobia, Nod factor, or other determinants.


Like [14*], this paper uses a biochemical approach to examine a Nod factor-binding site (NFBS2) isolated from *Medicago* cell cultures and the structural requirements of Nod factor for binding. Although recognition is mediated by both the lipid and oligosaccharide components of the Nod factor molecule, surprisingly, NFBS2 is not selective for the reducing end-sulfate group, which normally is essential for *Medicago* root hair deformation and nodulation.


This paper is a follow-up on the previous observation [45] made by this group that *Arabidopsis* has a receptor-like kinase (RLK) with an extracellular domain homologous to legume lectins. The previous paper [45] was the first report of a kinase with a lectin domain. The RLKs are glucosylated and localized to the plasma membrane fraction, but their function(s) is (are) unknown.