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Bacterial and plant glycoconjugates at the *Rhizobium*–legume interface

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Synopsis

Many classes of bacterial and plant glycoconjugate have been shown to be involved in establishing the *Rhizobium* root nodule symbiosis with peas (*Pisum sativum*). It was demonstrated, using techniques of molecular genetics, that a group of *Rhizobium* nodulation genes (*nod* genes) co-operate to synthesize a lipo-oligosaccharide signal molecule that specifically initiates nodule development on legume hosts. An additional gene function, encoded by *nodX*, has been found to extend the host range of *Rhizobium leguminosarum* bv. *viciae* to include nodulation of a pea mutant, cultivar Afghanistan; the *nodX* gene product specifies the addition of an acetyl group to the terminal *N*-acetylglucosamine residue at the reducing end of the pentasaccharide core of this signal molecule. Several other classes of bacterial glycoconjugate have also been shown by genetic analysis to be essential for normal nodule development and function: these include a capsular extracellular polysaccharide; lipopolysaccharide in the outer membrane; and cyclic glucans present in the periplasmic space. Potential functions for these glycoconjugates are discussed in the context of tissue and cell invasion by *Rhizobium*. Some plant components involved in symbiotic interactions have been identified by the analysis of nodule-specific gene expression (early nodulins). Several of the cDNA clones encoding these early nodulins specify proline-rich proteins that presumably correspond to cell wall glycoproteins or membrane arabinogalactan proteins. Other plant glycoconjugates have been identified using monoclonal antibodies as probes. A plant glycoprotein present in intercellular spaces has been identified as a component of the luminal matrix of infection threads. Because it attaches to the surface of bacteria and is itself susceptible to oxidative cross-linking, this glycoprotein may be involved in limiting the progress of microbial infections. Endocytosis of bacteria into the plant cytoplasm is apparently driven by direct interactions between the bacterial

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surface and the plasma membrane that is exposed within an unwalled infection droplet; glycoprotein and glycolipid components of the plant membrane glycocalyx have been defined using monoclonal antibodies. Differentiation of endosymbiotic bacteroids is preceded by differentiation of the plant-derived peribacteroid membrane which encloses the symbiosome compartment. Using a monoclonal antibody that identifies a group of plant membrane-associated, inositol-containing glycolipids, we have identified a very early marker for the differentiation of peribacteroid membrane from plasma membrane.

Introduction

The *Rhizobium*–legume symbiosis is characterized by the development of a new plant organ, the root nodule, with a specialized central infected tissue that provides a suitable micro-environment for biological nitrogen fixation [1]. Within each infected host cell, endosymbiosis is characterized by the development of a new form of quasi-organelle structure termed the 'symbiosome'. This comprises a differentiated form of *Rhizobium*, the 'bacteroid', enclosed by a plant-derived peribacteroid membrane, somewhat similar in structure to the host plasma membrane, but lacking the capacity to synthesize an associated cell wall and acquiring instead specialized transport functions that regulate the nutritional, osmotic and physiological status of the endosymbiont.

In terms of cell-to-cell signalling in nodule development (Fig. 1), there are several important issues which merit investigation, and in which the *Rhizobium*–legume symbiosis may serve as a model for the discovery of more general truths concerning the nature of plant development and of plant–microbe interactions. How is the nodule initiated? What sustains the process of nodule development and the accompanying

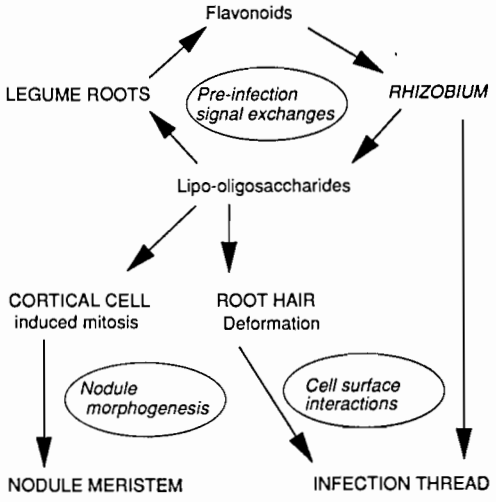

Fig. 1. Diagram showing three phases of cell-to-cell signalling involved in legume nodule development. (A) Pre-infection signal exchanges; (B) nodule organogenesis; (C) tissue and cell invasion by *Rhizobium*.

process of cell differentiation? What forces and intercellular signals drive the process of tissue and cell invasion by *Rhizobium*? What distinguishes a symbiotic form from a pathogenic form of host–microbial interaction?

One way to answer these questions has been to use genetics, because the analysis of malfunctions in plant and bacterial mutants that are symbiotically defective can tell us much about signal exchanges that are involved in normal nodule development [2,3]. Another approach has been to use the techniques of cell biology, either to investigate the phenomenon of tissue-specific gene expression [4,5], or to use monoclonal antibodies as probes to build up a picture of the antigens on the differentiating plant and bacterial cell surfaces at successive stages of nodule development. This last approach has illuminated the particular role of cell surface glycoconjugates in nodule development.

Our experimental system involves *R. leguminosarum* bv. *viciae*, which has been very well characterized genetically [2,6]. Among its hosts are the small-seeded, hairy vetch *Vicia hirsuta* (convenient for cytological analysis) and the garden pea *Pisum sativum* (convenient for biochemical analysis). Ever since the days of Mendel, the pea has been a classic plant for genetic analysis, and at least 30 genes affecting symbiotic development have been described [7]. In the present chapter, we will briefly review what has been learned about plant–microbial signal exchange from an analysis of pea mutants, and then we will examine the role of cell surface glycoconjugates in the process of tissue and cell invasion by *Rhizobium*.

Host plant genetics

The symbiotically defective mutants that have been isolated in pea have either been derived by screening for natural variation in field isolates or, more commonly, they are the product of mutagenesis with either chemicals or irradiation [7,8]. Examples of the five major phenotypic classes of symbiotically defective mutant are given in Table 1. Each of these classes provides some interesting clues concerning the nature of cell-to-cell signalling during nodule development.

The non-nodulating mutants either fail to respond to the bacterial lipo-oligosaccharide signalling molecule, or the response of root-hair cells is abnormal, resulting in stunted or distorted root hairs or the early abortion of the *Rhizobium*-induced infection thread [9]. Such mutants may either lack the receptor system for bacterial lipo-oligosaccharide signal molecules, or lack the mechanisms for transducing these signals into an effective host-cell response. The occurrence of several mutants with deformed and distorted root hairs indicates that reorganization of cell wall growth, presumably involving reorganization of the cytoskeleton, is an essential precondition for infection thread development. A fascinating subclass of these non-nodulating mutants is a group (involving several different loci) where the phenotype is pleiotropic, preventing establishment of the vesicular arbuscular mycorrhizal symbiosis as well as of the symbiosis with *Rhizobium* [10]. Two possible inferences can be drawn from this phenotype: either that both symbionts have a common system for suppressing the normal host defence system which prevents invasion by micro-organisms (and that this suppression system is inoperative in the mutant phenotype) or, alternatively, that the two symbionts make use of a common, non-disruptive system of cell and tissue invasion. Such a system might involve the formation of transcellular tunnels sheathed by plant

Table 1. Examples of symbiotically defective pea mutants.

Non-nodulating
Most mutants fail to respond to lipo-oligosaccharide signal molecules
Some mutants show abnormal root hair growth
Many mutants are also Myc$^-$ i.e. non-mycorrhizal
Strain-specific nodulation
Pea cultivar Afghanistan is *sym2 sym2* (homozygous recessive), i.e. only responds to acetylated form of lipo-oligosaccharide
Low frequency of nodulation
sym5 — mutant is ethylene hypersensitive
Non-fixing mutants (Fix$^-$)
sym13 — no nitrogenase induction by bacteroids, premature senescence
Sprint-2 Fix$^-$ — no nitrogenase, multiple bacteroids per symbiosome
Supernodulating mutants (nitrate-tolerant symbiosis)
nod3 — phenotype is root controlled
sym28 — phenotype is shoot controlled

References to each of these classes of mutant are given in the text.

cell wall and plant cell membrane, whose organized deposition is, in some way, controlled by the plant cytoskeleton.

A second group of pea mutants (derived both from natural variants and from induced mutagenesis) are mutants showing strain specificity for nodulation. The best-studied example is that of pea cultivar Afghanistan, which is resistant to nodulation by most strains of *R. leguminosarum* bv. *viciae*. The resistance character by which cultivar Afghanistan differs from normal cultivated garden peas corresponds to a single recessive Mendelian allele [11,12]. In the case of pea species, the evolutionary origin and centre of divergence is thought to be the eastern Mediterranean region, and it was found that certain field isolates of *R. leguminosarum* derived from soils in the Middle East were capable of nodulating effectively with cultivar Afghanistan, as well as with normal garden peas. One of these strains (designated TOM, a field isolate from Tomask in Turkey) was subjected to genetic analysis to investigate the basis for its extended host-range character (Table 2). It was found that the particular nodulation characteristics of TOM could be co-transferred to other strains of *R. leguminosarum* by transfer of the symbiotic plasmid (P*sym*) which also carries all the normal nodulation genes [13]. It was subsequently established, both by mutagenesis and by gene-cloning experiments, that the gene conferring the extension of host-range character in strain TOM was an extra gene (termed *nodX*) with no counterpart in a normal strain of *R. leguminosarum* [14–16]. When the *nodX* gene was sequenced, it was deduced, on the basis of motifs found in its predicted amino acid sequence, that the protein was likely to function as an acetyltransferase. Furthermore, in collaboration with Dr R.W. Carlson (University of Georgia, U.S.A.), we have recently demonstrated that strains of *R. leguminosarum* bv. *viciae* carrying the *nodX* gene synthesize a chemically modified form of lipo-oligosaccharide which carries an additional acetyl substitution on the terminal reducing sugar of

Table 2. Nodulation of peas by strains of *R. leguminosarum*.

R. leguminosarum strain or genotype	Pea cultivar Afghanistan *sym2 sym2*	Cultivated garden pea *Sym-2 Sym-2*
TOM field isolate (contains *nodX*)	+	+
300 field isolate (lacks *nodX*)	−	+
300 Psym$^-$	−	−
300 Psym$^-$ + Psym (TOM)	+	+
300 + *nodX*	+	+

nodX is an extra gene from strain TOM with no counterpart in strain 300 or other strains of *R. leguminosarum* bv. *viciae*. The DNA sequence of the *nodX* gene indicates homology with acetyltransferase. Lipo-oligosaccharide isolated from TOM or 300 *nodX* carried an additional acetyl group on the reducing sugar of the penta-glucosamine core.

the penta-glucosamine backbone (Fig. 2). Thus it appears that acetylation of the bacterial signal molecule (by the NodX gene product) is required to overcome the host-resistance character associated with the homozygous recessive genotype of cultivar Afghanistan. Now it will be interesting to discover the function of the dominant allele, Sym2, that is present in normal garden peas. Could it be that this plant gene product also serves to convert the unacetylated bacterial oligosaccharide (synthesized by strains of *R. leguminosarum* lacking *nodX*) into a form that is active in plant tissue? Perhaps the *Sym2* gene also encodes an acetyltransferase equivalent in function to the bacterial NodX gene product — but such possibilities are still only speculations at present.

A third group of symbiotically defective pea mutants corresponds to a phenotypic class that gives poor nodulation and frequently a temperature-sensitive nodulation phenotype. One such mutant is *sym5* [17,18]. This mutant has recently been shown to be hypersensitive to ethylene, reminding us that the process of nodule initiation and development is peculiarly sensitive to ethylene, far more so than other aspects of plant development, such as root growth [19]. It is interesting to note that ethylene is often synthesized by plant cells in response to stress, and hence its involvement in the control of nodule development is particularly intriguing [20].

A fourth general class of symbiotically defective phenotype, includes all mutants that create nodules in which nitrogen fixation does not take place. Some of these mutants show abnormalities in the process of nodule development. For example, in Sprint-2 Fix$^-$, surface contacts between intracellular bacteroids and the peribacteroid membrane are apparently altered so that the bacteroids are enclosed in groups of a dozen rather than singly, as in the wild-type symbiosis [21]. In another Fix$^-$ mutant, *sym13*, morphological development of bacteroids is normal but nitrogenase is not induced, presumably as a result of the failure of an essential plant-derived signal or metabolic transport function [22]. Interestingly, in such Fix$^-$ mutants, the bacteroids senesce prematurely, implying that the host plant has a surveillance system that can act to close down unproductive nodules that have been colonized by rhizobia, but where

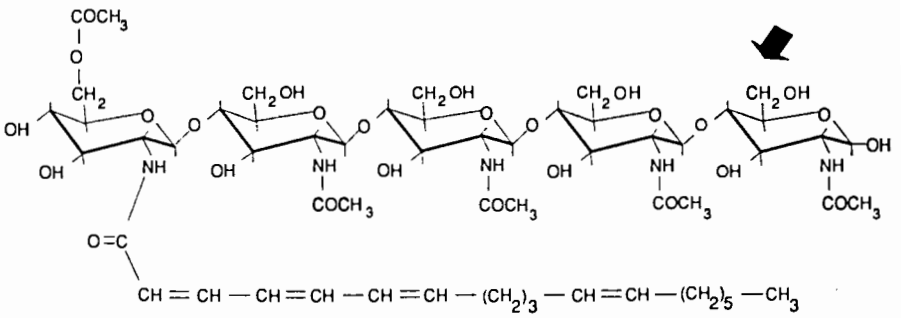

Fig. 2. Chemical structure of the lipo-oligosaccharide signalling molecule encoded by the nodulation genes of *R. leguminosarum* bv. *viciae*. This compound specifically initiates the development of nodules in pea roots by causing cortical cell divisions. The molecule has an oligosaccharide core, comprising four or five sugar residues of *N*-acetylglucosamine; this is a common feature of lipo-oligosaccharides synthesized by all species of *Rhizobium*. In each species, a different polyunsaturated fatty acid is attached to the amino group of glucosamine at the non-reducing end of the oligosaccharide chain; this confers one element of host specificity on the molecule. Other components of host specificity are an acetyl substitution on the terminal non-reducing sugar and a variety of possible substitutions on the terminal reducing sugar (but not shown on the molecular form illustrated here). In *R. leguminosarum* strain TOM, the action of the *nodX* gene product confers an additional acetyl substitution to the reducing sugar of the pentasaccharide core, as indicated by the arrowhead, thereby extending host range.

no nitrogen fixation results: such bacteria would be more accurately described as pathogens rather than as symbionts.

The fifth and final class of pea nodulation mutants are the so-called supernodulating mutants. These mutants were originally identified because their nodule development was not suppressed by growth of plants in high levels of fixed nitrogen (nitrate-tolerant symbiosis). In addition, these mutants have far more nodules on their roots than is normal [9,23]. They appear to be defective in a feedback control system that acts systemically to control nodule number (or nodule mass) on the whole plant. Furthermore, it has been ascertained from root–shoot grafting experiments, that in some of these lines the mutant phenotype is expressed in root tissue, whereas in others the phenotype is expressed by the shoot tissue, implying that both roots and shoots are involved in the mediation of this whole-plant control system.

Bacterial cell surface glycoconjugates

Recent genetic and biochemical analysis has shown that at least four kinds of *Rhizobium*-derived glycoconjugate molecule are essential for tissue and cell invasion by *Rhizobium*. These are (i) the diffusible lipo-oligosaccharide 'Nod-factor', which alone

can stimulate root-hair curling and cortical cell division [6]; (ii) acidic extracellular polysaccharide (EPS), which is a Ca^{2+}-gelling succinoglycan that encapsulates rhizobial cells and is apparently essential for infection thread initiation and development [24]; (iii) lipopolysaccharide (LPS), the major component of the bacterial outer membrane, which is essential for proper infection thread development, bacterial release into host cells and the proper development and functioning of nitrogen-fixing bacteroids [25,26]; and (iv) periplasmic cyclic glucan polymers, which may function in osmotic adaptation to the endophytic environment [27]. The initiation of an infection thread in pea root-hair cells involves the participation of live bacteria carrying appropriate EPS and LPS components in their cell wall.

Our work has focused on the role of bacterial lipopolysaccharide and its possible interaction with plant cell surface components at successive stages of pea nodule development, both in the early stages of infection thread development and subsequently during the differentiation of nitrogen-fixing bacteroids within the symbiosome compartment. The importance of LPS is suggested by the fact that mutants with a variety of modifications in the structure and biosynthesis of their LPS macromolecules are unable to establish a normal nitrogen-fixing symbiosis [26]; these mutants induce the development of abnormal root nodules on peas and other legumes [28–30]. We have analysed the development of these nodules and the fate of the LPS-defective mutant bacteria within them by using monoclonal antibodies and cytochemical techniques. The mutants fell into three general classes: severe mutants, inducing an 'empty nodule' phenotype; moderately severe mutants, inducing a delayed nodule development and reducing nitrogen fixation to less than 5% of the rate for wild-type nodules; and mildly disabled mutants, which only slightly impaired the normal processes of nodule development and nitrogen fixation.

The most severe LPS-defective mutant failed to invade nodule tissue. Inoculation of pea seedling roots with strain B659 induced the development of empty nodule-like structures with peripheral vasculature, a rudimentary endodermis and a central uninfected tissue which secreted quantities of an extracellular matrix glycoprotein that accumulated in the intercellular spaces [31]. However, in the absence of invading bacteria, no infection thread structures were seen.

The second group of LPS-defective mutants induced nodules in which only a small proportion of the central nodule tissue was colonized by bacteria. Consequently, much of the central tissue was occupied by uninfected parenchyma, particularly underneath the nodule endodermis. Despite the abnormal development of infection threads and the relatively low number of infected host cells in these nodules, the infected host cells still induced leghaemoglobin production and the endosymbiotic bacteria enclosed by peribacteroid membranes also induced the synthesis of nitrogenase. The presence of both of these proteins was detected by immunostaining with specific antisera. The appearance of nodule cells invaded by LPS-defective mutants was found to be highly heterogeneous. Adjacent to viable infected cells with nitrogenase-containing bacteroids, other cells were found with clear signs of cytoplasmic disorganization and collapse. This suggests that the signal eliciting the host defence response was very localized.

The ultrastructural analysis of infected nodule cells also revealed clear differences between wild-type and LPS-defective bacteroids. The mutant bacteria were always released into the plant cytoplasm surrounded by a peribacteroid membrane; but, in

contrast to wild-type bacteroids which are normally Y-shaped, the bacteroids formed by mutant strains were usually highly branched and much larger in size. Moreover, in nodules formed by LPS-defective mutants, several bacteroids were commonly seen inside the same peribacteroid membrane envelope, whereas in wild-type nodules bacteroids were always individually enclosed. Mutant bacteroids also showed premature senescence and induced the formation of apparently lytic vesicles in the host-cell cytoplasm. From these observations of abnormal nodule development, it seems that LPS-defective mutants are a little closer to the borderline between symbiotic and pathogenic interactions with the host plant. We conclude that the correct LPS structure is essential for the avoidance of host defence responses and for the physiological adaptation of rhizobia to the endophytic environment.

Glycoconjugates in the plant intercellular matrix

Using the monoclonal antibody MAC 265 as a probe, we have identified a 95–110 kDa plant glycoprotein secreted into the lumen of infection threads as an early response to *Rhizobium* infection [32,33]. The glycoprotein (or family of glycoproteins) recognized by MAC 265 has recently been purified from pea nodules, using a procedure based on immunoaffinity chromatography, but the *N*-terminal sequence that has been obtained does not conform to any of the known classes of plant extracellular glycoproteins. Some indications are now emerging about the physical properties of the plant matrix glycoprotein and how it might interact with invading rhizobia in the lumen of infection threads.

We have recently demonstrated that the matrix glycoprotein is capable of attachment through ionic binding to the surface of rhizobial cells, derived either from free-living culture or isolated from nodules. This attachment occurs also with *Rhizobium* mutants lacking the outer (O-antigen) components of LPS, and also with mutants lacking the extracellular capsular polysaccharide (Fig. 3). Moreover, the binding of matrix glycoprotein to the bacterial surface was always stronger after the capsular polysaccharide had been removed by prior washing in a high-salt buffer. Thus, it seems probable that the capsular polysaccharide (and perhaps also the O-antigen components of LPS) may actually inhibit binding of matrix glycoprotein to the bacterial cell surface by masking the highly charged groups that lie closer to the surface of the bacterial outer membrane. It should also be noted that the attachment of matrix glycoprotein to the bacterial surface is not specific to *Rhizobium*: it occurs equally well with cultured cells of *Escherichia coli*. Therefore, it is conceivable that the matrix glycoprotein functions as part of a general antimicrobial defence system that entraps invading micro-organisms, preventing their further penetration into host tissues. In this case, the essential feature of a successful invasion might be the ability to escape entrapment by the intercellular plant matrix glycoprotein. On this model, the role of *Rhizobium* extracellular polysaccharide in infection thread growth in peas might be to provide a capsular sheath that masks the charged components on the bacterial surface from interaction with plant matrix glycoprotein: the outer (O-antigen) components of LPS might also have an enhancing role in this function, since mutants lacking these components show some abnormalities in infection thread growth (Fig. 4).

Support for the model that matrix glycoprotein functions in a microbial entrapment system comes from observation of the physical behaviour of the glyco-

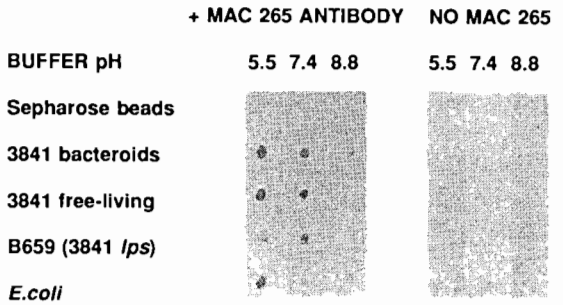

Fig. 3. Binding of plant matrix glycoprotein to the surface of bacteria, as revealed by immunostaining with MAC 265, a rat monoclonal antibody recognizing the glycoprotein. Bacterial cell suspensions (10^9 cells per ml) were incubated with crude matrix glycoprotein (derived from nodule homogenates) and diluted in buffer solutions (50 mM) containing dithiothreitol (5 mM) and adjusted to the pH values indicated. After incubation for 1 h, bacteria were re-isolated by centrifugation and washed extensively. Aliquots (1 μl) were immobilized on nitrocellulose sheets and probed with MAC 265 (left-hand sheet) or, as a negative control, with an irrelevant antibody (right-hand sheet), followed by peroxidase anti-(rat immunoglobulin) and chromogenic substrates.

protein under conditions of oxidative stress. It was found that the glycoprotein could only be purified from nodule homogenates in the presence of antioxidants (ascorbic acid and dithiothreitol). In high oxygen concentrations or in low protein concentrations the glycoprotein had a tendency to become insoluble. This process was enhanced by the introduction of peroxidase enzyme and hydrogen peroxide into the medium. It is well known that plant cells, particularly those that have been subjected to microbial attack, often respond to stress by inducing an oxidative burst [34]. If these oxidative conditions resulted in the intercellular glycoprotein becoming cross-linked and insolubilized, it is clear that this could also result in the entrapment of invading bacteria, thus limiting the progress of invasion. What is less clear, however, is the nature of the signals from the bacterium that might induce the host plant cells to respond by the generation of the oxidative burst. Perhaps ethylene is also an important component of the host defence response, since this gas is known to enhance the activity of peroxidases [35].

Plant glycoconjugates associated with the peribacteroid membrane

There is a very interesting transition which occurs during nodule development when rhizobia change from being in the extracellular (apoplastic) environment to being intracellular, having been engulfed by plasma membrane that is exposed in an unwalled infection droplet structure. The engulfment, or endocytosis, of rhizobia seems to involve a close surface interaction between unencapsulated rhizobia and the surface of the plasma membrane, which still carries an apparently typical glycocalyx composed of glycolipid and glycoprotein components [36]. Endocytosis is accompanied by the

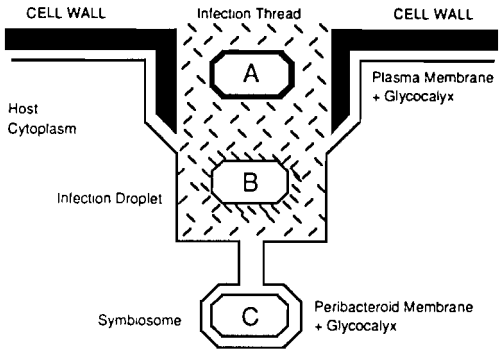

Fig. 4. Model for cell-surface interactions during tissue and cell invasion by *Rhizobium*. (A) The infection thread: bacteria are enclosed within a tubular ingrowth of the plant cell wall. A capsular sheath of extracellular polysaccharide cocoons the bacteria, which are embedded in a matrix of plant glycoproteins similar in composition to that found in intercellular spaces. (This is represented as hatched material, which is recognized by antibody MAC 265.) Close contact between the bacterial outer membrane and the plant cell membrane is prevented by the presence of bacterial capsule and the plant cell wall. (B) The infection droplet: synthesis of plant cell wall and bacterial capsule is reduced or inhibited. Plant matrix glycoprotein becomes attached to the bacterial cell surface. Subsequently, endocytosis occurs as a result of surface interactions between plant and bacterial membranes. (C) The symbiosome: after engulfment of bacteria, the plant matrix glycoprotein is apparently excluded from the symbiosome compartment. The peribacteroid membrane retains a glycocalyx similar to that of the plasma membrane (recognized, for example, by antibody MAC 206). Subsequent division and differentiation of intracellular rhizobia leads to the development of nitrogen-fixing bacteroids. The ensheathing plant membrane, which acquires new material by fusion of vesicles from the Golgi and endoplasmic reticulum, becomes functionally specialized as the peribacteroid membrane. In pea nodules, the peribacteroid membrane divides in synchrony with dividing bacteroids so that only a single bacteroid is enclosed within each symbiosome unit.

apparent loss of the matrix glycoprotein from the bacterial surface and thus it is possible that the interaction between the bacterial surface and the matrix glyoprotein is replaced by a similar interaction with a component of the plant membrane glycocalyx [33]. Furthermore, as intracellular bacteria continue to divide, the accompanying peribacteroid membrane also divides concomitantly, implying continued surface interactions between plant and bacterial membrane surfaces. It is interesting to note that the function of the plasma membrane glycocalyx of plant cells has been postulated to involve some form of physical interaction with the plant cell wall [37]. However, although the peribacteroid membrane has a glycocalyx, there is no associated plant cell

wall, but only a bacterial cell wall with which the plant membrane might possibly interact.

As the infected cells of the nodule gradually mature, the peribacteroid membrane becomes differentiated by acquiring nodule-specific proteins (nodulins) and transport functions associated with the specialized metabolism of this nitrogen-fixing 'organelle'. These developmental changes proceed in phase with the progressive differentiation of the intracellular rhizobia into nitrogen-fixing bacteroid forms. A monoclonal antibody has recently been isolated that recognizes a new class of plant glycolipid membrane antigen. *In situ* immunostaining of pea nodule sections with this antibody reveals that the corresponding antigen is always present on the plasma membrane but it disappears from the peribacteroid membrane at a precise point in nodule differentiation which slightly precedes the induction of leghaemoglobin. Thus, loss of the glycolipid antigen from the peribacteroid membrane coincides with differentiation of this membrane and the enclosed bacteria into nitrogen-fixing organelles. This glycolipid seems to be a marker for a very early developmental switch from a 'juvenile' to a 'mature' form of peribacteroid membrane, which may be very relevant to the subsequent course of symbiosome differentiation.

After extraction with various organic solvents, the glycolipid antigen from pea nodule membranes has been examined by thin-layer chromatography. Because a similar glycolipid component can be identified in extracts of carrot cell membranes, carrot cell suspension cultures have been used for incorporation studies with radiolabelled sugar precursors. It has thus been demonstrated that the glycolipid incorporates label from inositol and glucosamine. Moreover, the presence of phosphate is indicated by the fact that the antibody reacts in dot immunoassays with phosphatidylinositol monophosphate (PIP). Although further work is needed to characterize the chemical structure of this membrane glycolipid from pea nodules, the current experimental evidence suggests that it belongs to one of the groups of inositol-containing glycolipids, most probably the glycosyl-phosphatidylinositols or the glycophosphosphingolipids. The properties of these groups of glycolipids have not been well characterized in plants. In animal systems, the glycosyl-phosphatidylinositols can act either as membrane anchors for Golgi-derived extracellular proteins or they can be cleaved as part of a signal transduction pathway, releasing diacylglycerol and inositol phosphoglycan as intracellular messengers [38]. Very little is known about the intracellular signals controlling differentiation of the peribacteroid membrane and of the consequent onset of nitrogen fixation. However, using a range of bacterial mutants in conjunction with molecular probes for *in situ* cytological analysis, it is now possible to embark on this investigation by analysing the synthesis and metabolism of this family of inositol-containing membrane glycolipids.

Conclusions

Rhizobium invades host cells and tissues as a result of a reorganization of plant cell wall growth which is initiated by a host-specific signal, the lipo-oligosaccharide, that is synthesized and secreted by the appropriate *Rhizobium* strain. This leads to the development of an intracellular tunnel, the infection thread, that traverses the host plant cell from one side to the other and provides a channel for the entry of rhizobial cells

embedded in an extracellular matrix material secreted by the plant. Some of the components involved in cell surface interactions have been identified using monoclonal antibodies as molecular probes. The role of LPS in cell and tissue invasion was investigated by examining pea nodules induced by mutants of *R. leguminosarum* with defects in LPS structure and biosynthesis. We conclude that the correct LPS structure is essential for invasion of plant cells and tissues, for avoidance of host defence responses and for physiological adaptation to the endophytic microenvironment. Differentiation of endosymbiotic bacteroids is preceded by differentiation of the plant-derived peribacteroid membrane which encloses the symbiosome compartment. Using monoclonal antibody probes, we have identified a number of glycolipid and glycoprotein components of the peribacteroid membrane that may be involved in surface interactions with bacteroids. In addition, we have identified a group of plant membrane-associated, inositol-containing glycolipids, which serve as a very early marker for the differentiation of peribacteroid membrane from plasma membrane. Further analysis of plant–microbe signal exchanges during nodule development is likely to depend heavily on the analysis of plant mutants as well as bacterial mutants that are symbiotically defective.

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