Rhizobial lipo-oligosaccharides: answers and questions

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Mini review

Rhizobial lipo-oligosaccharides: answers and questions

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Abstract

Rhizobium bacteria produce certain lipo-oligosaccharides (modified chitin oligomers) after induction of nodulation (*nod*) gene transcription by the host plant. The function of the rhizobial *nod* genes in the biosynthesis of these lipo-oligosaccharides, focusing on their host specific aspects, is discussed. The lipo-oligosaccharides can elicit various responses in the host plants, like the formation of pre-infection threads and nodule meristems. Speculating on their function in plant morphogenesis the question is raised: do the rhizobial lipo-oligosaccharides resemble unknown plant signal molecules?

Introduction

Bacteria belonging to the genera *Rhizobium*, *Bradyrhizobium* and *Azorhizobium*, collectively called rhizobia [51], are able to invade the roots (or adventitious roots) of their host plants where they trigger the formation of a new organ called the root nodule (reviewed in [6]). In these root nodules a differentiated form of the rhizobia, the bacteroid, is able to fix nitrogen into ammonia, which can then be utilized by the plant. The host-specific aspect of this symbiosis is very pronounced and has led to the definition of cross-inoculation groups in which the bacterial species are classified according to their group of host plants. Examples of such cross-inoculation groups are *R. leguminosarum* biovar. *viciae* with peas and vetch as hosts, *R. leguminosarum* biovar. *trifolii* with clovers as hosts and *R. meliloti* with alfalfa and sweet clovers as hosts. At least two steps of signal exchange ('questions and answers') between plant and bacteria appear to be involved in the determination of host-specific nodulation. In the first step, flavonoids excreted by the plant induce the transcription of the bacterial nodulation genes (called *nod* or *nol* genes) (Fig. 1). The host specificity of this induction process involves the bacterial NodD protein, a transcriptional regulatory protein that presumably interacts directly with the flavonoids [19, 39]. The process in which NodD activates transcription has been the subject of intensive study (reviewed in [22, 34]). In the second step, the bacterium, by means of the *nod* or *nol* genes, produces lipo-oligosaccharide signals (Fig. 2) that induce various root responses which are discussed in this review. The common nodulation genes *nodA, nodB* and *nodC* are essential for biosynthesis of the lipo-oligosaccharides (also called Nod metabolites), consistent with their pivotal role in the infection and nodulation process. Several *nod* genes which were shown to be involved in the determination of host specificity of nodulation also appear to be involved in the production of lipo-oligosaccharide signals. Most noteworthy are the genes *nodQ* and *nodH*, which are major determinants of host specificity in *R. meliloti* [12, 24], and the gene *nodE*, which was shown to be

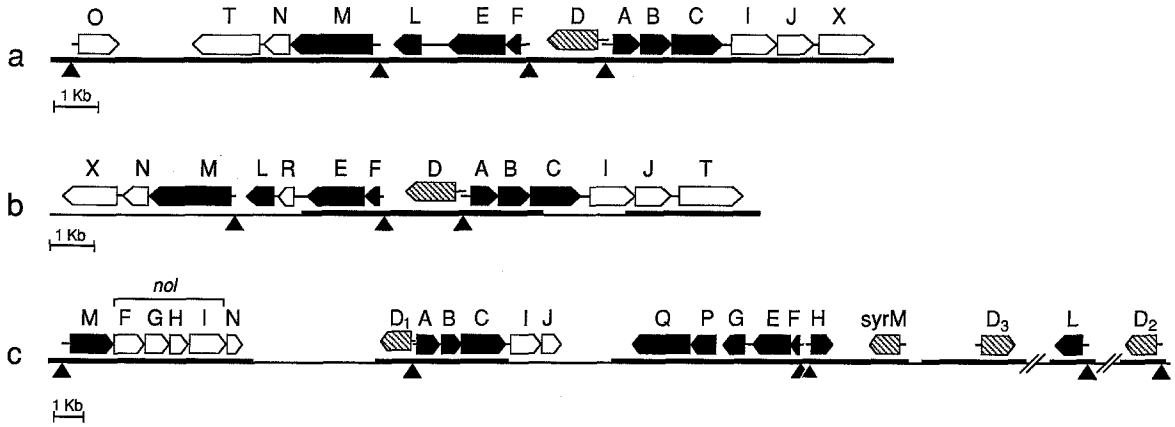

Fig. 1. The *nod* and *nol* genes of *R. leguminosarum* biovar. *viciae* (a), *R. leguminosarum* biovar. *trifolii* (b) and *R. meliloti* (c). Arrows indicate the position of the open reading frames of the *nod* and *nol* genes. In the genetic map thick lines are used to indicate that the nucleotide sequence of these regions has been published. Black triangles are used to indicate the position of the *nod* boxes (part of the inducible *nod* promoters). Black arrows are used to indicate the *nod* gene products which are presumed to be involved in the biosynthesis of the lipo-oligosaccharides. Shaded arrows indicate the transcriptional regulatory *nodD* and *syrM* gene products. The map of the *nod* genes of *R. leguminosarum* biovar. *viciae* is given for the Sym plasmid pRL1JI except for the translational product of the gene *nodX* which is not present on pRL1JI but has been reported in *R. leguminosarum* biovar. *viciae* strain TOM. The map of the *nod* genes of *R. leguminosarum* biovar. *trifolii* is given for the Sym plasmid pRtrANU843. The map of the *nod* and *nol* genes of *R. meliloti* is based on the data reported for *R. meliloti* strains 2011 and AK41. The presented data are according to the references [12, 13, 15, 22, 24].

the major determinant of the difference between the host specificities of the *R. leguminosarum* biovars *viciae* and *trifolii* [40].

Structures of lipo-oligosaccharides

After the initial discovery of lipo-oligosaccharides produced by *R. meliloti* strain 2011 [23] the structures of other lipo-oligosaccharides produced by this strain and by two other rhizobial strains have been reported (Fig. 2). Uniformly they consist of an oligosaccharide backbone of β-1,4-linked *N*-acetyl-D-glucosamine varying in length between three and five sugar units. To the nitrogen group of the non-reducing sugar moiety a fatty acid group is attached, the structure of which is variable. The presence of other substitutions is dependent on the rhizobial strain. In the case of the structures reported for the two *R. meliloti* strains 2011 and AK41 a sulphate group is present on carbon-6 of the reducing terminal sugar [23, 36]. In the case of *R. leguminosarum* biovar. *viciae* strain RBL5560 an *O*-acetyl group is present on carbon-6 of the non-reducing terminal sugar while the sulphate group is absent [41]. In *R. meliloti* strain 2011 a minor fraction of the lipo-oligosaccharides also contained an additional *O*-acetyl group on carbon-6 of the non-reducing terminal sugar [45]. These molecules were not observed in strain AK41 [36]. However, it should be noted that in these two *R. meliloti* strains the expression of the *nod* genes was artificially increased, by introducing cloned *nod* genes in a high copy number, in order to obtain sufficient quantities of compound for analytical purposes. It was shown that the relative amount of *O*-acetylated compounds produced by strain 2011 was dependent on the way in which overproduction was achieved [30].

Important differences have been observed in the nature of the fatty acyl chain. In the case of *R. meliloti* strain 2011, the lipo-oligosaccharides invariably contain a C16 acyl group with two double bonds (C16:2), between carbons 2 and 3 and carbons 9 and 10 [23]. Also in *R. meliloti* strain AK41 this acyl moiety is present on the major products. However, a minor class of molecules contains a different acyl chain with an extra *trans*-

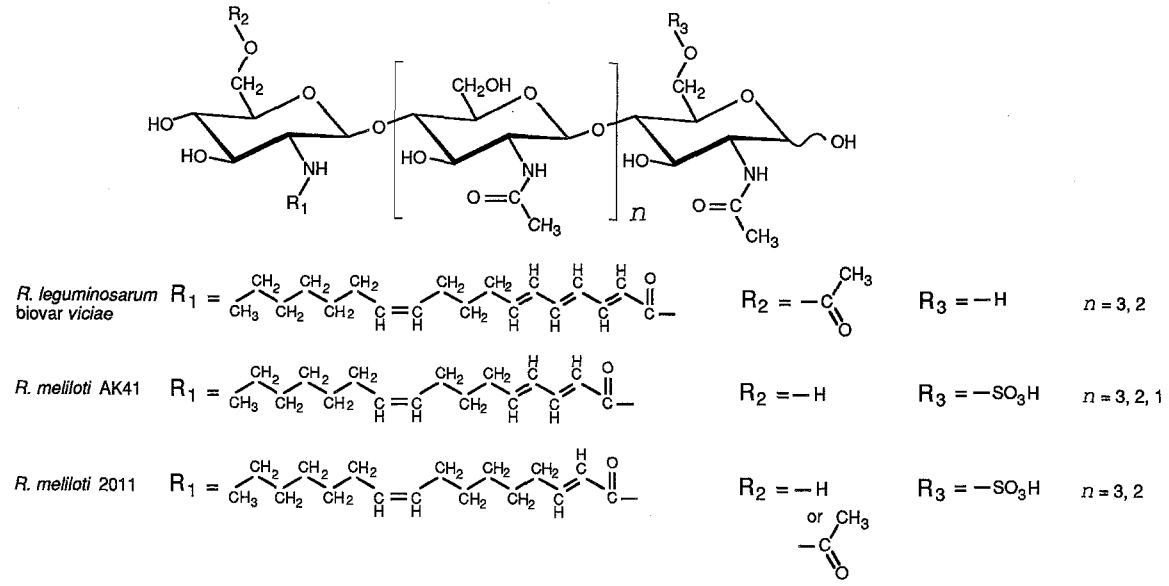

Fig. 2. Structures of lipo-oligosaccharides produced by three *Rhizobium* strains. Structures are according to Lerouge *et al.* [23] for *R. meliloti* strain 2011, Spaink *et al.* [41] for *R. leguminosarum* biovar. *viciae* strain RBL5560 and Schultze *et al.* [36] for *R. meliloti* strain AK41. In *R. leguminosarum* biovar. *viciae* one class of lipo-oligosaccharides contains *cis*-vaccenic acid (Fig. 3) instead of the fatty acid group shown. In strain AK41 the majority of the signals contains the same fatty acid group as is shown for strain 2011 [36]. In the nomenclature used for the rhizobial Nod metabolites a roman numeral following the species indication (e.g.Rlv for *R. leguminosarum* biovar. *viciae*) refers to the number of glucosamine units (n + 2) and a term in parenthesis indicate fatty acyl carbon number and degree of unsaturation (R_1), *O*-acetylation (R_2) and sulphation (R_3), respectively, e.g.: NodRm-IV(C16:2,Ac,S).

conjugated double bond at position 4 (Fig. 2) [36]. In *R. leguminosarum* biovar. *viciae* strain RBL5560 the nature of the fatty acyl chain is also variable. However, in this case the major fatty acyl moieties are C18 chains which either contain four or one double bonds. Three double bonds of the C18:4 chain are *trans*-conjugated to the carbonyl group while the fourth *cis*-double bond is located at the same position as in the C18:1 acyl moiety (*cis*-vaccenic acid) [41].

Based on the similarities between the published lipo-oligosaccharide structures a nomenclature has been introduced which is outlined in the legend to Fig. 2. This nomenclature is also applicable to the recently characterized lipo-oligosaccharide produced by *R. leguminosarum* biovar. *trifolii* (A. Aarts, pers. comm.), *Bradyrhizobium* (R. Carlson, H.P. Spaink and G. Stacey, unpublished results), broad-host-range *Rhizobium* strain NGR234 ([12] and N. Price, pers. comm.), *R. tropici* strains (J. Folch and H.P. Spaink, unpublished results; E. Martinez, pers. comm.) and *Azorhizobium caulidonans* (M. Holsters, pers. comm.). The basic structures of the lipo-oligosaccharides produced by these strains are similar to the ones described in Fig. 2, but can contain novel additional decorations.

Biochemical function of the Nod proteins

Insight into the biochemical function of several Nod proteins involved in the biosynthesis of lipo-oligosaccharides has been obtained in four ways: (1) comparison of the structures of lipo-oligosaccharides produced by wild-type *Rhizobium* strains, strains which contain mutations in the *nod* genes or which contain cloned *nod* genes; (2) comparison of the deduced Nod protein sequence with other proteins of known function and subsequently further comparison of their characteristics (e.g. expected cellular localization, prosthetic groups); (3) determination of *in vitro* enzymatic activity or (4) complementation of *Escherichia coli* mutants disturbed in a biochem-

ical pathway. The Nod proteins which were indicated to be involved in biosynthesis of the lipo-oligosaccharides are shown as black arrows in Fig. 1.

Until now the first approach has yielded most of the information on the role of Nod proteins in the biosynthesis of lipo-oligosaccharides. Mutations in the *nodABC* genes completely prevented production of the lipo-oligosaccharides in all rhizobial strains tested [23, 41, 42]. In *R. leguminosarum* biovar. *viciae* a mutation in the *nodE* gene prevented the production of the lipo-oligosaccharides which contain the highly unsaturated fatty acid moiety. A mutation in the *nodL* gene resulted in the lack of the *O*-acetyl groups on all produced lipo-oligosaccharides [41]. A mutation in the *nodM* gene resulted in a quantitative decrease of the production of lipo-oligosaccharides which were identical in structure [41, 42]. This leaky phenotype might be due to the presence of a chromosomal counterpart of *nodM* [25]. Mutations in other *nod* genes of *R. leguminosarum* biovar. *viciae* had no observable effect on the production of lipo-oligosaccharides [42]. These results are consistent with the observation that, besides *nodABC*, no other Sym plasmid-localized genes (Fig. 1) are required for the production of a basic lipo-oligosaccharide molecule [41]. In *R. meliloti* a mutation in the *nodH* gene resulted in the lack of the sulphate group on all produced lipo-oligosaccharides. Mutations in the *nodP* and *nodQ* genes resulted in the production of a mixture of sulphated and unsulphated factors [30]. Since *R. meliloti* contains at least one other copy of the *nodPQ* genes these could have partially complemented for the mutations [37, 12].

The information obtained by comparison of Nod protein sequences with other proteins of known function is summarized in Table 1. For most of the Nod proteins shown to be important for lipo-oligosaccharide production (Fig. 1, black arrows), homologies have been found which fit with a function in lipo-oligosaccharide biosynthesis. The only exceptions are the NodA and NodB proteins which showed no significant similarities with any published protein sequence.

The NodC protein is similar in sequence to a domain of all known classes of chitin synthases suggesting that it functions as an *N*-acetylglucosaminyltransferase that synthesizes the oligosac-

Table 1. Predicted biochemical function of Nod proteins presumed to be involved in the biosynthesis of lipo-oligosaccharides on basis of sequence homology.

Nod protein	Predicted function[1]	Ref.
NodC	*N*-acetylglucosaminyltransferase (a)	[5, 7, 32]
NodE	β-ketoacylsynthase (b)	[4, 15, 18, 46]
NodF	acyl carrier protein (c)	[4, 15, 18, 17]
NodG	dehydrogenase (NADH-dependent) (d)	[12]
	β-ketoacylreductase (NADPH-dependent) (e)	[38]
NodH	sulphotransferase (f)	[30]
NodL	acetyltransferases (g)	[15, 2]
NodM	D-glucosamine synthase (h)	[1, 25]
NodP	ATP sulphurylase subunit (i)	[37]
NodQ	ATP sulphurylase subunit (j)	[37]

[1] These predicted functions are based on homologies with the following proteins (in most cases the protein sequence is deduced from the nucleotide sequence of the genes): a, chitin synthases from yeast (CHS1, CHS2 and CSD2) and various fungi and cellulose synthase (BcsA) from *Acetobacter xylinum*; b, β-ketoacylsynthases from *E. coli* (FabH and FabB involved in biosynthesis of fatty acids, see Fig. 3) and varions *Streptomyces* species (e.g. encoded by *tcmI* ORF1 and *graI* ORF1 involved in biosynthesis of polyketide antibiotics); c, ACP from various prokaryotes and eukaryotes; d, ribitol dehydrogenase (*Klebsiella aerogenes*), alcohol dehydrogenase (fruit fly) and glyceraldehyde 3-phosphate dehydrogenase (*Thermus aquaticus*); e, β-ketoacylreductases from avocado (partial protein sequence) and various *Streptomyces* species (e.g. ActIII from *S. coelicolor*); f, steroid sulphotransferases from mammals; g, LacA, CysE and LpxA from *E. coli*; h, GlmS from *E. coli*; i, CysD from *E. coli*; j, CysN from *E. coli*.

charide backbone of the lipo-oligosaccharides [7]. NodC has been shown to be membrane-located [21]. Comparison of the hydrophobicity plots of NodC with chitin synthases [5, 7] and cellulose synthase [32] suggests that these are all membrane proteins with a characteristic transmembrane helix in the C-terminal region for anchoring it into the membrane. The NodM protein was shown to have sequence homology with glucosamine synthase (encoded by *glmS*) [25] and therefore could have a function in the production of glucosamine, an obvious precursor for the synthesis of the oligosaccharide backbone. This predicted biochemical function of NodM is supported by the observation that the *nodM* gene was able to complement an *E. coli glmS*$^-$ mutation [1].

The sequence homology of NodF with acyl carrier proteins (ACP) and of NodE with various β-ketoacylsynthases [4, 15] leads to the hypothesis that they function in the synthesis of the C18:4 fatty acid in *R. leguminosarum* biovar. *viciae* (Fig. 2). This hypothesis is further supported by the observation that NodF contains a 4′-phosphopantetheine prosthetic group [17] which can function as carrier for acyl chains during fatty acid biosynthesis or transfer. Based on the present knowledge of the biosynthesis of fatty acids in prokaryotes a simple model is presented which could explain the function of NodF and NodE in the synthesis of the C18:4 fatty acid (Fig. 3). In this model it is assumed that NodE protein, by using regular fatty acid intermediates as substrates (which cannot be used by the household β-ketoacyl synthases), competes with normal fatty acid synthesis. Interestingly, this model can also be used to explain the synthesis of the C16:3 lipid present on a class of lipo-oligosaccharides of *R. meliloti* strain AK41 (Fig. 2 and Fig. 3, second intermediate in panel B). The *R. meliloti* NodG protein, based on its sequence homology to β-ketoacyl reductases (arrow 2 in Fig. 3) and the location of its gene in the same operon as *nodF* and *NodE*, is likely to be involved in the synthesis of the C16:2 fatty acid (Fig. 2). However, speculation on its function is made difficult by its homology to two different classes of enzymes (Table 1). In this respect it can be noted that the activity of (FAD-dependent) acyl-CoA dehydrogenase, the first step in oxidative degradation of fatty acids, yields the C16:2 fatty acid as an intermediate. Therefore it would be interesting to know whether NodG is also homologous to this enzyme.

The homology of the NodL protein with several acetyl transferases [15, 2] is consistent with its indispensable role in the addition of the *O*-acetyl moiety. Likewise, the homology of

Table 2. Effects of rhizobial lipo-oligosaccharides on the roots of leguminous plants.

Effect and abbreviation	Nod metabolite[1]	Test organism	Ref.
Root hair deformation (HAD)	NodRlv(*), NodRm(C16:2)	*Vicia*	[23, 41]
	NodRm(C16:2,S) and NodRm (C16:3,S)	*Medicago* and *Melilotus*	[23, 36]
Induction of root hair initiation (HAI)	NodRm(C16:2)	*Vicia*	[30]
Depolarization of root hair membrane potential	NodRm(C16:2,S)	*Medicago*	[16]
Thick and short roots (TSR)	NodRlv(*)	*Vicia*	[47, 41]
Production of additional flavonoids (INI)	NodRlv(C18:4,Ac)	*Vicia*	[28, 29]
Induction of nodulin gene (ENOD12, ENOD5) expression	NodRlv(C18:4,Ac)	*Pisum*	[33, 20]
Induction of pre-infection threads (PIT)	NodRlv(C18:4,Ac)	*Vicia*	[49]
Outer cortical cell outgrowths	NodRlv(C18:4,Ac)	*Vicia*	[49]
Induction of nodule meristems (NOI)	NodRlv(C18:4,Ac)	*Vicia*	[41, 49]
	NodRm(C16:2,S)	*Medicago*	[45]

[1] Nod metabolites which were active are indicated leaving out the indication for the sugar chain length (Fig. 2) which was shown to be not essential to induce any of the listed effects. An asterisk is used to indicate that it has been reported that no specific decorations are required.

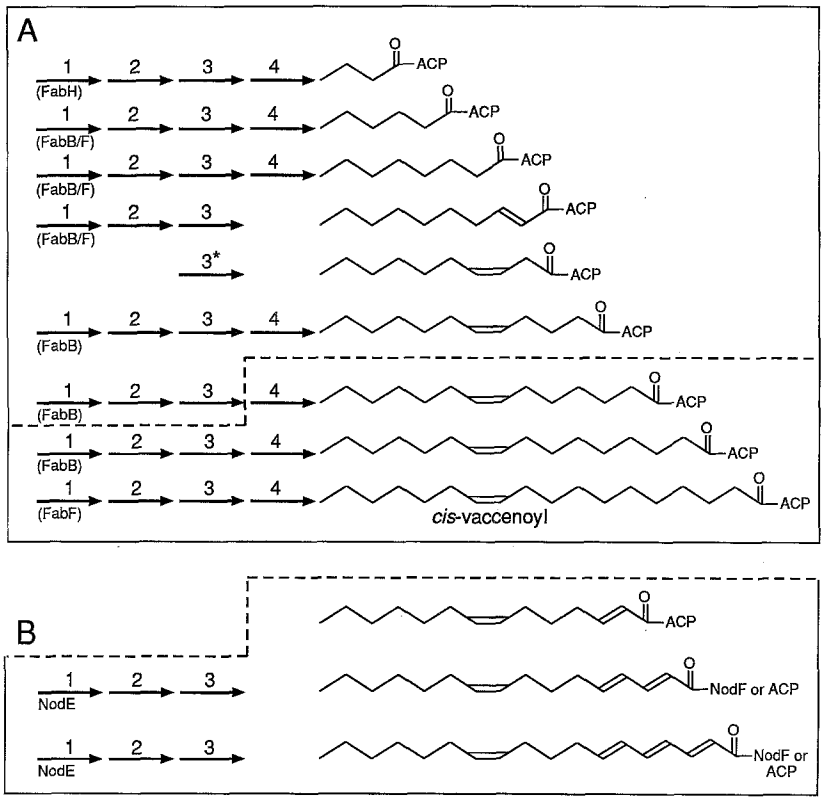

Fig. 3. A model which compares the synthesis of the most common bacterial mono-unsaturated fatty acid, *cis*-vaccenic acid, and the synthesis of the highly unsaturated, NodE-dependent, fatty acid of *R. leguminosarum* biovar. *viciae*. In panel A, a model of the synthesis of *cis*-vaccenic acid is given based upon the present knowledge of the fatty acid biosynthesis in *E. coli* [8, 18, 46]. Explanation of the numerals: **1**, condensation reaction (releasing one CO_2); **2**, β-ketoacyl reduction (NADPH-dependent); **3**, dehydration (releasing one H_2O); **4**, enoyl reduction (NADPH-dependent); **3***, isomerization (this is done by the second activity of the enzyme 3-hydroxydecanoyl-ACP-dehydrase). FabB, FabF and FabH are the described β-ketoacylsynthases of *Escherichia coli* [8, 46]. By replacing the indicated bottom segment of panel A with panel B, the hypothesized situation when the NodF and NodE proteins are expressed in *Rhizobium* is described. The assumption that normal ACP is substituted for NodF, by an assumed transferase activity of NodE, is optional and not an essential part of the model (although it is a possible mechanism for the avoidance of step 4 because enoyl-ACP-reductase might not recognize enoyl-NodF intermediates).

NodH with sulphotransferases [30] is consistent with its indispensable role in the addition of the sulphate moiety. The NodP and NodQ proteins share homology with the *E. coli* ATP sulphurylase subunits CysD and CysN, respectively, indicating that they could function in the production of an activated form of sulphate which is required for the sulphotransferase activity [37]. *In vitro* studies have demonstrated that the NodP and NodQ proteins indeed have ATP sulphurylase activity [37]. For NodL, *in vitro* studies have also been performed showing that it has acetyltransferase activity (G. Bloemberg, pers. comm.).

Nothing is known about how the lipo-oligosaccharides are transported to the outside of the cell. The NodI protein, encoded by the *nodABCIJ* operon (Fig. 1), shares sequence homology with a large family of traffic ATPases and has therefore been proposed to be involved in the secretion of the lipo-oligosaccharides [15]. However, transposon mutations in the *nodI* gene did not have a quantitative effect on the amount of lipo-oligosaccharides present in the growth medium under laboratory conditions [42]. Recent results, using multicopy plasmid containing cloned *nod* genes indicate that *nodI* and *nodJ* could have an influence on the excretion of lipo-oligosaccharides [43].

Effects of the Nod metabolites on the plant

The effects of rhizobial extracellular factors on the roots of host and non-host plants have been studied for many years and recently the role of the *nod* genes in these effects was investigated (reviewed in [48, 12]). After the discovery of the lipo-oligosaccharides, their effect on the roots has been studied intensively and has also yielded various novel phenotypes (Table 2). These discernible effects of the lipo-oligosaccharides on the plant root emphasize the importance of this new class of signal molecules as a tool to study plant morphogenesis.

The phenotype which is most obviously relevant for the rhizobium-plant symbiosis is the induction of the nodule meristem. It has been shown that purified lipo-oligosaccharides of *R. meliloti* and *R. leguminosarum* biovar. *viciae* are able to induce meristems in the inner cortex of the roots of *Medicago sativa* and *Vicia sativa*, respectively, which are indistinguishable from the nodule meristems in the first stage of normal nodule organogenesis [41, 45]. These nodule meristems were observed frequently by external application of wild-type signals, in concentrations as low as 1 nM [45] and 50 nM [41], to the plant growth medium. In the case of *Medicago* the nodule meristems were capable of developing further into full-grown nodules which had anatomical and histological features of genuine rhizobium-induced nodules, such as apical meristems and peripheral vascular bundles and endodermis [45]. In *Vicia* this was never observed but instead the development of the meristem stopped at a stage in which small outgrowths of the roots were externally visible ([41, 49] and A. van Brussel, pers. comm.).

In addition to their role in nodule meristem induction, certain effects of the lipo-oligosaccharides have been observed which suggest that they are also involved in the infection process. Van Brussel *et al.* [49] have shown that the lipo-oligosaccharides isolated from wild-type *R. leguminosarum* biovar. *viciae* bacteria are able to induce a phenotype in *Vicia* roots which they called pre-infection thread formation. These pre-infection threads are characterized by the occurrence of outer cortical cells in which the nucleus and cytoplasm has moved to the centre of the cell, as if the cell started the process of cell division. Rows of such cells in which the central cytoplasms zones are radially aligned, give the impression of strands which cross the outer cortex. The formation of these structures, which were also observed after infection with *R. leguminosarum* biovar. *viciae* bacteria, always precedes the formation of infection threads and are therefore named pre-infection threads [49]. It was speculated that these pre-infection thread structures are the first step in infection thread formation [49]. Another indication that lipo-oligosaccharides are involved in the infection process comes from the observation that the transcription of certain nodulin genes which are differentially and transiently expressed during the infection process is also induced by the lipo-oligosaccharides [33, 26, 20]. The effects observed after treatment of the host plant roots with rhizobial lipo-oligosaccharides indicate that they trigger a whole programme of plant developmental processes which does not require the presence of bacteria. This notion is supported by the observation that a certain proportion of wild-type *Medicago sativa* plants can spontaneously develop genuine root nodules in the absence of rhizobia or Nod signals [44].

An intriguing observation is that the lipo-oligosaccharides of *R. leguminosarum* biovar. *viciae* are able to elicit the production and excretion of additional species of flavonoids in *Vicia sativa* roots (INI, see Table 2) [28]. It was suggested that the induction of INI is at the level of transcriptional regulation of plant genes involved in flavonoid biosynthesis [29]. Since the additional species of flavonoids are efficient inducers of the NodD-dependent *nod* gene expression the INI effect could be interpreted as a positive feedback mechanism ('a continuous interchange of answers and questions').

The structural requirements for the lipo-oligosaccharides to induce the effects on plant roots varies tremendously depending on the host plant. But even for the induction of root hair deformation, being amongst the least specific ef-

fects, it was shown that oligomers of chitin (in Fig. 2: R_1, R_2 and R_3 being hydrogen atoms) were insufficient for induction [41]. In *Medicago* the sulphate group (Fig. 2) is required for root hair deformation and meristem induction [45, 30]. In contrast, the presence of the sulphate moiety on the *R. meliloti* signals prevents the induction of root hair deformation on the non-host plant *Vicia* [23, 30]. The induction of INI, nodule meristems and pre-infection threads in *Vicia* apparently requires the presence of the highly unsaturated (C18:4) fatty acid and the *O*-acetyl moiety (Fig. 2) [41, 49]. Derivatives of the *R. meliloti* lipo-oligosaccharides in which the double bonds of the acyl chain were removed by hydrogenation were also not able to induce nodule meristems in *Medicago* [45]. These data correlate well with the infection and nodulation phenotypes of rhizobia which are mutated in the *nodE* (specific fatty acid moiety), *nodL* (*O*-acetyl moiety) and *nodH* (sulphate moiety) genes (see [12]). However, it should be noted that the effects of the mutations are not as absolute as in the *in vitro* situation. For example, in the case of a *nodE* mutant of *R. leguminosarum* biovar. *viciae* a delayed and reduced nodulation is still observed [14], and the effect of a mutation in *nodH* on nodulation is very much dependent on the laboratory conditions [27]. In the former case it has been shown that the *nodO* gene, which encodes an excreted protein which is homologous to haemolysin, partially complements for the function of the *nodE* gene in nodulation [11, 14]. This complementation can explain the discrepancy between the *in vitro* and *in vivo* situation, but the molecular basis is still completely unknown.

Speculation on the role of lipo-oligosaccharides

The discernible effects of the rhizobial lipo-oligosaccharides on plant gene expression and morphogenesis raise various questions as to their role in plant development. Do host-specific receptors for the lipo-oligosaccharides exist? It is unknown whether the host-specific decorations of the lipo-oligosaccharides are recognized by specific receptors or if these aspects just serve as a means to reach a more general receptor (e.g. for transport or protection against degradation). Is the effect on cellular dedifferentiation in the inner cortex a direct effect on the cell division control mechanism? This is argued against by several observations which suggest that root nodule formation is the result of a change of the cytokinin-auxin hormone balance (reviewed in [12, 26, 50]). Do rhizobial lipo-oligosaccharides resemble unknown plant signal molecules? It has been shown that oligosaccharides can have various effects on plant morphogenesis [9]. The resemblance of the bacterial signals to chitin together with recent indications for the involvement of chitinases in plant development [10], gives new ground for discussion. Indeed the occurrence of various classes of chitinases in plants has been the cause for much speculation as to their function and possible substrates in the plant (see [10, 43] and references given therein). The results of Benhamou [3] indicate that chitin derivatives occur in secondary plant cell walls of various plant species. Recently, Spaink *et al.* [43] have obtained results which suggest that lipophilic molecules which can be degraded by chitinase are present in uninfected *Lathyrus* plants. If chitin derivatives occur in plants it could also explain the changed growth phenotype of tobacco plants transformed with either *nodA* or *nodB* [35]. As reported by Sandal and Marcker [31] and Bulawa [7] the NodC protein is very similar to the DG42 protein of *Xenopus laevis*, both in direct sequence homology (26 % identity over 520 amino acids) and hydrophobicity pattern. Although the function of the DG42 protein is unknown, it has been shown that it is differentially expressed during embryogenesis. Regarding these observations it is indeed tempting to speculate that chitin derivatives have an essential signal function in higher organisms.

Acknowledgements

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Note added in proof

Sanjuan *et al.* (Sanjuan J, Carlson RW, Spaink HP, Bhat UR, Barbour WM, Glushka J, Stacey G: Proc Natl Acad Sci USA 89, in press) have reported the structure of lipo-oligosaccharides produced by *Bradyrhizobium japonicum*, a distant relative of *Rhizobium* which nodulates soybean. The structure is similar to those of the lipo-oligosaccharides produced by various *Rhizobium* strains but is different in that it contains a 2-*O*-methylfucose on carbon-6 of the reducing terminal *N*-acetylglucosamine.

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