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MINI REVIEW

Nod factor structures, responses, and perception during initiation of nodule development

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The onset of nodule development, the result of rhizobia–legume symbioses, is determined by the exchange of chemical compounds between microsymbiont and leguminous host plant. Lipo-chitooligosaccharidic nodulation (Nod) factors, secreted by rhizobia, belong to these signal molecules. Nod factors consist of an acylated chitin oligomeric backbone with various substitutions at the (non)reducing-terminal and/or nonterminal residues. They induce the formation and deformation of root hairs, intra- and extracellular alkalinization, membrane potential depolarization, changes in ion fluxes, early nodulin gene expression, and formation of nodule primordia. Nod factors play a key role during nodule initiation and act at nano- to picomolar concentrations. A correct chemical structure is required for induction of a particular plant response, suggesting that Nod factor–receptor interaction(s) precede(s) a Nod factor-induced signal transduction cascade. Current data on Nod factor structures and Nod factor-induced responses are highlighted as well as recent advances in the characterization of proteins, possibly involved in recognition of Nod factors by the host plant.

Key words: legume nodulation/lipo-chitooligosaccharide/Nod factor receptor/signal transduction/three-dimensional Nod factor structure

Introduction

Nodulation (Nod) factors are key signal molecules that play a pivotal role during initiation of nodule development and bacterial invasion (Broughton *et al.*, 2000; Perret *et al.*, 2000). They are produced by rhizobia, including the genera *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium*. The recent discovery of nodulating *Methylobacterium* sp. (Sy *et al.*, 2001) and *Burkholderia* sp. (Moulin *et al.*, 2001) that belong to the α- and β-subclass of the Proteobacteria, respectively, calls for expansion of the rhizobia with these latter bacterial genera. Rhizobia nodulate specific leguminous host plants and the nonlegume *Parasponia*. Such symbioses result in the formation of root nodules, new organs occupied by differentiated bacteria, that fix atmospheric nitrogen and provide it to their respective host plant, thereby promoting plant growth independently of the available soil nitrogen. Mature nodules are either of the determinate or indeterminate type (Crespi and Gálvez, 2000). Determinate nodules are formed on some tropical and subtropical legumes (e.g., soybean, bean) and are characterized by a round-shaped appearance, initiation of nodule primordia in the outer cortex, and meristematic activity that disappears early after nodule initiation. Oval-shaped, indeterminate nodules usually form on roots of temperate legumes (e.g., pea, alfalfa, vetch), nodule primordia initiate in the inner cortex, the meristematic activity is persistent, and the central tissue consists of a number of distinct zones (Crespi and Gálvez, 2000). *Medicago truncatula* (Bell *et al.*, 2001) and *Lotus japonicus* (Kawasaki and Murakami, 2000) are now considered the best model legumes. Their genomes are being sequenced to efficiently determine plant responses occurring during all stages of nodule development.

Nod factors consist of an oligomeric backbone of β-1,4-linked *N*-acetyl-D-glucosaminyl residues, N-acylated at the nonreducing-terminal residue (Kamst *et al.*, 1998) and thus are lipo-chitooligosaccharides (LCOs) (Dénarié *et al.*, 1996). Rhizobia synthesize populations of Nod factors that consist of two (in the case of *Rhizobium etli* CFN42; Poupot *et al.*, 1995), to approximately 60 (in the case of *R. galegae* HAMBI1207; Yang *et al.*, 1999) different individuals. Qualitative and quantitative aspects of Nod factor populations are strain-specific. The Nod factor structure differs in the number of GlcNAc residues present in the chitooligosaccharide backbone, in the nature of the fatty acyl group, and in the substituents at the nonreducing- and/or reducing-terminal residues. In a few cases, Nod factor substituents are found at nonterminal GlcNAc residues. Nod factor synthesis depends on the expression of nodulation (*nod*) genes, comprising the *nod*, *nol*, and *noe* genes. Recently, orthologs of the *nodA* gene, one of the key *nod* genes encoding an acyl transferase (Kamst *et al.*, 1998), have been discovered in symbiotic *Methylobacterium* sp. (Sy *et al.*, 2001) and *Burkholderia* sp. (Moulin *et al.*, 2001).

Major Nod factor–triggered responses include the formation and deformation of root hairs, intra- and extracellular alkalinization, membrane potential depolarization, changes in ion fluxes, induction of early nodulin gene expression, and formation of nodule primordia (Broughton *et al.*, 2000; Perret *et al.*, 2000). Detailed structure analyses of Nod factor populations produced by a variety of rhizobia and phenotypic studies with mutant rhizobia demonstrated the importance of Nod factor structures

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for causing particular responses. Nod factors act in concentrations as low as 10^{-9} to 10^{-12} M, and particular substituents protect against the Nod factor hydrolysis by enzymes of host plant origin. These observations, together with the fact that Nod factors preferentially migrate into root hair cell walls (Goedhart *et al.*, 1999), suggest that perception by (a) Nod factor receptor(s) may be an initial and essential requirement for Nod factor signaling.

This review summarizes the present data on Nod factor structures and Nod factor–induced responses during nodule initiation, focusing on the importance of the chemical structure for biological activity. Recent advances concerning the characterization of putative Nod factor receptors are highlighted.

Nod factor structures

A landmark in the *Rhizobium*–legume symbioses was the report on the structure of the Nod factors of *Sinorhizobium meliloti* by Lerouge *et al.* (1990). Currently, Nod factor populations produced by many rhizobial strains from different genera and geographical origins are known in detail (Figure 1; Table I).

Most Nod factors consist of a backbone of three, four, or five β-1,4-linked *N*-acetylglucosaminyl residues, N-acylated at the nonreducing-terminal residue by either a "common" fatty acid, such as vaccenic (C18:1) and stearic (C18:0) acid, or by a (poly)unsaturated fatty acid, such as C20:1 (*Mesorhizobium loti* NZP2213) or C18:4 (*R. leguminosarum* bv. *viciae* A1) (Table I). Often, *N*-methyl, *O*-acetyl, and *O*-carbamoyl groups are found at the nonreducing-terminal residue and L-fucosyl, 2-*O*-Me-fucosyl, 4-*O*-Ac-fucosyl, acetyl, and sulfate ester at the reducing-terminal residue (Table I).

It is interesting that a few Nod factors have exceptional lengths or types of oligosaccharide backbone or substitutions at the terminal residues (Table I). Some Nod factors of *M. loti* NZP2213 have a dimeric chitooligosaccharidic backbone (Olsthoorn *et al.*, 1998) and some of *Rhizobium* sp. GRH2 consist of six GlcNAc residues (López-Lara *et al.*, 1995b), whereas *Sinorhizobium fredii* USDA191 can synthesize a pentameric Nod factor whose middle GlcNAc residue is replaced by a glucosyl group (Bec-Ferté *et al.*, 1996). The C-1 position of the reducing-terminal GlcNAc residue of some Nod factors produced by *Bradyrhizobium elkanii* USDA61 and *R. tropici* CIAT899 are substituted with a glycerol (Carlson *et al.*, 1993; Stokkermans *et al.*, 1996) and a mannosyl (Folch-Mallol *et al.*, 1996) moiety, respectively. In part of the Nod factor population synthesized by *M. huakuii* Ra5, the CH_3CO group of the reducing-terminal GlcNAc residue is replaced by a CH_2OHCO group (Yang *et al.*, 1999). Also the 3-*O*-S-2-*O*-Me-fucosyl and 4-*O*-Ac-2-*O*-Me-fucosyl residues found at C-6 of the reducing-terminal residue are unique for *Sinorhizobium* sp. NGR234 Nod factors (Price *et al.*, 1992, 1996). Furthermore, the reducing-terminal GlcNAc can be transformed into an open ring structure, such as an acetylated glucosaminitol group in the case of *Rhizobium* sp. BR816 (Snoeck *et al.*, 2001). Finally, some Nod factors carry modifications at the GlcNAc residue proximate to the nonreducing-terminal residue, for example, an α-1,3-linked fucosyl group at C-3 in *M. loti* NZP2213 (Olsthoorn *et al.*, 1998), an acetyl group at C-3 in *R. galegae* HAMBI1207 (Yang *et al.*, 1999), or an acetyl group at C-6 in *Rhizobium* sp. BR816 (Snoeck *et al.*, 2001). Diglycosylated Nod factors produced by *S. teranga* bv. *sesbaniae* ORS604 (Lorquin *et al.*, 1997a), *S. saheli* ORS611 (Lorquin *et al.*, 1997a), and *Azorhizobium caulinodans* ORS571 (Mergaert *et al.*, 1997), carrying both a fucosyl and an arabinosyl group at the reducing-terminal residue, have been observed only in rhizobia that nodulate the tropical legume *Sesbania rostrata* (Table I).

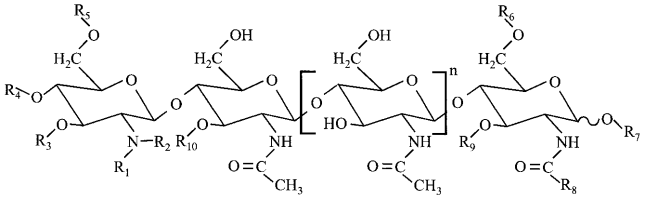

Fig. 1. General structure of Nod factors produced by rhizobia. For the identity of the substitutions (R_1–R_{10}) and the oligomerization degree (*n*), see Table I.

Nod factor responses and their structural requirements

Most commonly, rhizobia enter the host plant's root tissue through the intracellular infection thread mode, after they have colonized root hair tips (Kijne, 1992). Based on the developmental stage of root hairs, three zones from the root tip toward the older part of the root are defined: zone I with growing root hairs, located near the root tip; zone II or "susceptible zone," carrying root hairs that terminate growth; and zone III, which consists of mature, fully grown root hairs. Generally, root hairs belonging to the susceptible zone respond to Nod factor–producing rhizobia, with root hair deformation and curling as a result. Small confinements appear that are formed by the curl, called shepherd's crooks, in which rhizobia are entrapped. At these sites, rhizobia enter the root hair intracellularly, via an inward-growing infection thread. These infection threads guide the rhizobia to the newly developing nodule primordia, which are localized foci of cortical cell divisions. Subsequently, rhizobia become internalized in the cytoplasm of young plant cells. Many early nodulation events are induced by Nod factors. These plant responses occur at the epidermis, cortex, and pericycle and are summarized in Table II. Some of the early responses in epidermis and cortex are described in more detail.

Epidermis

Alkalinization. The structural requirements of Nod factors for the alkalization response are given in Table II. In root hairs of *Medicago sativa* (alfalfa), one of the first responses to Nod factor treatment is an increase in intracellular, cytosolic pH with more than 0.2 U that lasts for 5 min and starts as early as 15 s after addition of 10^{-7} M NodSm-IV (C16:2,S) (Felle *et al.*, 1996). When 10^{-9} M NodSm-IV (C16:2,S) is added to alfalfa roots, a rapid but transient alkalinization at the root surface is observed, peaking 3 min after Nod factor addition (Felle *et al.*, 1998). Chitotetraose and chitopentaose, but not chitotriose and chitohexaose, also give a weak intracellular alkalinization when added at a final concentration of 10^{-7} M. Chitooctaose (10^{-7} M) can mimic extracellular alkalinization, but the pH increase is followed by an acidification, which is not observed after

Table I. Overview of Nod factor structures and their specific substitutions

Rhizobial strain	Nod factor substitutions[a,b]											Reference(s)*
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	*n*[c]	
***Azorhizobium* sp.**												
A. caulinodans ORS571	C18:1,C18:0 C16:0[d,e]	Me	H	H	Cb,H	Fuc,Ara,H	H	Me	Ara,H	H	1, 2	1
***Bradyrhizobium* sp.**												
Bradyrhizobium strains[z]	C18:1,C18:2 C16:0,C16:1	Me,H	Cb,H	H	Cb,H	3-*O*-S-2-*O*-MeFuc H	H	H	H	H	2	2
B. aspalati bv. *carnosa*[a′]	C16:1,C16:0 C18:1,C19:1cy C18:0,C20:1	Me,H	H	Cb	Cb	H	H	H	H	H	0, 1, 2	3
B. elkanii USDA61	C18:1,C16:0	Me,H	Cb,Ac[f] H	Cb,Ac,H	Cb,Ac,H	2-*O*-MeFuc,Fuc	Gro,H	Me	H	H	1, 2	4, 5
B. japonicum USDA110	C18:1[g]	H	H	H	H	2-*O*-MeFuc	H	Me	H	H	2	6
B. japonicum USDA135	C18:1,C16:0,C16:1[h]	H	H	H	Ac,H	2-*O*-MeFuc	H	Me	H	H	2	4
***Mesorhizobium* sp.**												
Mesorhizobium sp. N33	iso-C15:0,iso-C17:1,iso-C17:0[c′]	H	H	H	H	S	H	Me	H	H[q]	2	7
M. loti E1R[i]	C18:1,C18:0	Me	H	Cb	H	4-*O*-AcFuc	H	Me	H	H	2	8
M. loti JRL501	C18:1[b′]	Me	Cb[f],H	Cb[f],H	Cb[f],H	Fuc,4-O-AcFuc	H	Me	H	H	2	9
M. loti NZP2037	C18:1,C18:0	Me	Cb,H[f]	Cb	Cb,H[f]	4-*O*-AcFuc	H	Me	H	H	2	8
M. loti NZP2213	C16:0,C16:1,C18:0 C18:1,C20:0,C20:1 C22:1,OH-C18:1[j]	Me,H	Cb,H	H	Ac[k],H	4-*O*-AcFuc Fuc,H	H	Me	H	Fuc[l],H	–1, 0 1, 2	10
M. huakuii Ra5[m]	C18:4[n],C18:1[o]	H	H	H	H	S,H	H	Me CH_2OH	H	H	0, 1, 2	11
***Rhizobium* sp.**												
Rhizobium sp. BR816[p]	C18:0,C18:1	Me	H	H	Cb	S	H	Me	H	H[q]	2	12
Rhizobium sp. GRH2	C16:0,C18:0,C18:1 C20:1[j]	Me,H	H	H	H	S,H	H	Me	H	H	1, 2, 3	13
Rhizobium sp. ORS1001	C18:0,C18:1	Me	Cb[f],H	Cb[f],H	Cb[f],H	S,H	H	Me	H	H	2	14
R. etli CE3	C18:0,C18:1	Me	H	Cb	H	4-*O*-AcFuc	H	Me	H	H	2	15
R. etli CFN42	C18:1	Me	Cb[f],H	Cb,H	Cb,H	4-*O*-AcFuc	H	Me	H	H	2	16
R. galegae HAMBI1207	C14:0,C16:0,C16:1 C18:0,C18:1,C18:2 C18:3,C18:4,C20:2 C20:3,C18:0/3-OH C20:1/3-OH[r]	H	H	H	Cb,H	H	H	Me	H	Ac,H	1, 2	11
R. leguminosarum bv. *trifolii* ANU843	C16:0,C16:1,C18:0 C18:1,3OH-C16:0 3OH-C14:0,C18:2 3OH-C18:0	H	Ac[s],H	Ac,H	Ac,H	H	H	Me	H	H	0, 1, 2	17–19
R. leguminosarum bv. *trifolii* LPR5045	C18:0,C18:1,C18:3 C20:1,C20:2,C20:3 C20:4,C18:0-OH	H	H	H	Ac	H	H	Me	H	H	0, 1, 2	20, 21
R. leguminosarum bv. *viciae* A1	C16:0,C16:1,C18:0 C18:1,C18:2,C18:3 C18:4,C18:1-OH[j]	H	H	H	Ac,H	Ac,H	H	Me	H	H	0, 1, 2	22
R. leguminosarum bv. *viciae* RBL5560	C18:4,C18:1,C18:0 C16:0,C16:1[n]	H	H	H	Ac	H	H	Me	H	H	1, 2	20, 23
R. leguminosarum bv. *viciae* TOM	C18:4,C18:1	H	H	H	Ac[t]	Ac[u],H	H	Me	H	H	1, 2	24
R. tropici CFN299	C18:1	Me	H	H	H	S,H	H	Me	H	H	2	25
R. tropici CIAT899	C16:0,C16:1,C18:0 C18:1,C20:0,C20:1 [j]	Me,H	H	H	H	S,H	Man H	Me	H	H	1, 2	26
***Sinorhizobium* sp.**												
Sinorhizobium sp. NGR234	C18:1,C16:0 C16:1, C18:0 C18:2	Me	Cb,H	Cb,H	Cb,H	3-*O*-S-2-*O*-MeFuc 3-/4-*O*-Ac-2-*O*-MeFuc 2-*O*-MeFuc	H	Me	H	H	2	27–29

Table I. Overview of Nod factor structures and their specific substitutions

Rhizobial strain	Nod factor substitutions[a,b]											Reference(s)[*]
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	*n*[c]	
S. fredii 257DH4	C16:0,C18:1	H	H	H	H	2-*O*-MeFuc	H	Me	H	H	0, 1, 2	30
S. fredii HH103	C16:0,C16:1 C18:0,C18:1[v]	H	H	H	H	2-*O*-MeFuc,Fuc	H	Me	H	H	0, 1, 2	31
S. fredii USDA191[w]	C16:0,C16:1 C18:0,C18:1	H	H	H	H	2-*O*-MeFuc,Fuc	H	Me	H	H	0, 1, 2	30
S. fredii USDA257	C18:1	H	H	H	H	2-*O*-MeFuc,Fuc	H	Me	H	H	0, 1, 2	32
S. meliloti RCR2011	C16:1,C16:2,C16:3 C18-C26(ω-1)-OH[x]	H	H	H	Ac,H	S	H	Me	H	H	0, 1, 2	33–37
S. saheli ORS611	C16:0,C18:1	Me	Cb[f],H	Cb[f],H	Cb[f],H	Fuc[y],Ara,H	H	Me	Ara,H Fuc[y]	H	1, 2	38
S. teranga bv. *acaciae* ORS1073	C16:0,C18:0,C18:1	Me	Cb[f],H	Cb[f],H	Cb[f],H	S,H	H	Me	H	H	2	14
S. teranga bv. *sesbaniae* ORS604	C16:0,C18:1	Me	Cb[f],H	Cb[f],H	Cb[f],H	Fuc[y],Ara,H	H	Me	Ara,H Fuc[y]	H	1, 2	38

Abbreviations: Ac, acetyl; Ara, arabinosyl; Cb, carbamoyl; Fuc, fucosyl; Gro, glycerol; Man, mannosyl; Me, methyl; S, sulfate ester.

[a]R_1-R_{10} and n refer to Figure 1 with a schematic representation of a general Nod factor.

[b]One of the first conventions concerning Nod factor nomenclature was proposed by Roche *et al.* (1991a). After the term Nod, referring to nodulation factors, the bacterial source was indicated followed by a roman numeral corresponding to the number of β-1,4-linked GlcNAc residues present in the chitooligosaccharide backbone. In a second bracketed caption, the fatty acid was given, according to the general lipid nomenclature, if the Nod factor contained a fatty acyl chain that differed from those present on Nod factors produced by the wild-type strain (Roche *et al*., 1991a). For instance, a tetrameric Nod factor produced by *S. meliloti* carrying a C16:2 fatty acid and substituted with an acetyl group and a sulfate ester at the nonreducing-terminal and the reducing-terminal residue, respectively, was indicated as NodRm-IV (Ac,S), but its hydrogenated derivative was indicated as NodRm-IV (Ac,S) (C16:0). This nomenclature was slightly modified by Spaink (1992), who suggested the use of one bracketed caption representing the fatty acyl chain in any case, followed by the different substitutions listed clockwise, starting from the fatty acyl chain. The latter wild-type Nod factor was then indicated as NodRm-IV (C16:2,Ac,S). Finally, Heidstra and Bisseling (1996) proposed to reorganize the term in parenthesis by separating the substitutions at the nonreducing- and reducing-terminal residues by the length and degree of unsaturation of the fatty acyl chain, giving, for instance, NodRm-IV (Ac,C16:2,S) for the previous example. The latter nomenclature will be used throughout this work. When the exact species name of a particular member of the rhizobia has not been defined yet, the rhizobial source will be indicated with part of the strain name (for example, NodNGR factors for *Sinorhizobium* sp. NGR234). Exceptionally, Nod factors produced by *A. caulinodans* ORS571 will be referred to as NodARc instead of NodAc factors to avoid possible confusion with the symbolic representation of an acetyl group.

[c]Oligomerization degree.

[d]Indication of possible substitutions, a comma should be interpreted as "or."

[e]Without specifications, the fatty acids correspond to common fatty acids as for example C18:1, vaccenic acid (C18:1Δ11).

[f]The exact position of Cb or Ac groups at the nonreducing-terminal residue is not known.

[g]C18:1Δ11, vaccenic acid as reexamined by Stacey *et al.* (1994).

[h]Not enough material was available to localize the double bond in C16:1.

[i]*M. loti* E1R produced the same Nod factors strains as CIAM1801, NZP2235, and NZP2238.

[j]No indications were presented about localization of double bonds in unsaturated fatty acids.

[k]The Ac group indicated at C-6 can be at C-4 as well.

[l]The fucosyl residue substituted at the GlcNAc proximal to the nonreducing-terminal residue is linked via an α-1,3 bond.

[m]Nod factors produced by *M. huakuii* strains Ra98 and 7653R are identical to those produced by strain Ra5.

[n]C18:4Δ2,4,6,11.

[°]Only traces of Nod factors carrying vaccenic acid were detected.

[p]The reducing-terminal GlcNAc residue can be an *N*-acetyl glucosaminitol residue substituted with no, one, or two additional acetyl groups.

[q]A population of Nod factors contains an acetyl group at the C-6 position of the GlcNAc residue proximate to the nonreducing-terminal residue.

[r]C16:1 (Δ7), C18:1 (Δ7; Δ11; Δ2), C18:2 (Δ2,11 + other isomers), C18:3 (Δ2,4,11), C18:1 (Δ11/3-OH), C20:2 (Δ2,13 + other isomers), and C20:3 (Δ2,4,13).

[s]Some Nod factors appeared to be diacetylated, but no further indications about the position of the second Ac group were given.

[t]Fully unacetylated Nod factors may also be present, although it is not known whether these are present in the cells or caused by a deacetylation during the extraction procedure.

[u]Only pentameric Nod factors are 6-*O*-acetylated at the reducing-terminal residue.

[v]C16:1(Δ9).

[w]A unique Nod factor, annotated as NodSf-V-Glc (C18:1, MeFuc), was synthesized from which the GlcNAc residue in the middle of the pentameric backbone was replaced by a glucosyl residue.

[x]C18-C26(ω-1)-OH included C18, C20, C22, C24, C26 hydroxylated species, C16:1 (Δ9), C16:2 (Δ2,9), and C16:3 (Δ2,4,9).

[y]Fuc or Ara are either at C-3 or C-6, respectively, in monoglycosylated Nod factors; in diglycosylated Nod factors Fuc is at C-6 and Ara at C-3.

[z]These *Bradyrhizobium* strains were isolated from *Acacia albida* (Ferro *et al*., 2000).

[a′]Moulin *et al.* (2001) demonstrated that *B. aspalati* bv. *carnosa* is taxonomically more related to *Burkholderia* sp., belonging to the β-subclass of the Proteobacteria.

[b′]Description of the Nod factor population is incomplete. Nod factors with other fatty acids may be present in the population (Niwa *et al*., 2001).

[c′]13-Methyl-tetradecanoic acid, iso-C15:0; 15-methyl-hexadecanoic acid, iso-C17:0; 15-methyl-2-hexadecanoic acid, iso-C17:1.

[*]1, Mergaert *et al.* (1997); 2, Ferro *et al.* (2000); 3, Boone *et al.* (1999); 4, Carlson *et al.* (1993); 5, Stokkermans *et al.* (1996); 6, Sanjuan *et al.* (1992); 7, Poinsot *et al.* (2001); 8, López-Lara *et al.* (1995a); 9, Niwa *et al.* (2001); 10, Olsthoorn *et al.* (1998); 11, Yang *et al.* (1999); 12, Snoeck *et al.* (2001); 13, López-Lara *et al.* (1995b); 14, Lorquin *et al.* (1997b); 15, Cárdenas *et al.* (1995); 16, Poupot *et al.* (1995); 17, Philip-Hollingsworth *et al.* (1991); 18, Philip-Hollingsworth *et al.* (1995); 19, Orgambide *et al.* (1995); 20, Spaink *et al.* (1995); 21, van der Drift *et al.* (1996); 22, Ovtsyna *et al.* (1999); 23, Spaink *et al.* (1991); 24, Firmin *et al.* (1993); 25, Poupot *et al.* (1993); 26, Folch-Mallol *et al.* (1996); 27, Price *et al.* (1992); 28, Price *et al.* (1996); 29, Berck *et al.* (1999); 30, Bec-Ferté *et al.* (1996); 31, Gil-Serrano *et al.* (1997); 32, Bec-Ferté *et al.* (1994); 33, Lerouge *et al.* (1990); 34, Truchet *et al.* (1991); 35, Roche *et al.* (1991b); 36, Schultze *et al.* (1992); 37, Demont *et al.* (1993); 38, Lorquin *et al.* (1997a).

Table II. Nod factor–induced responses in various systems

Response	Test plant	Compound[a]	Conc.[b]	N/S[c]	+/–[d]	Time[e]	Reference
Epidermis							
Formation of new root hairs (Hai)							
	V. sativa	NodSm-IV (Ac,C16:2,S)	10^{-9}	N	–		Roche *et al.* (1991a)
		NodSm-IV (C16:2,S)	10^{-9}	N	–		Demont-Caulet *et al.* (1999)[f]
		NodSm-IV (Ac,C16:2)	10^{-11}	N	+		
		NodSm-IV (C16:2)	10^{-11}	N	+		
	Acacia cyanophylla/ A. melanoxylon	NodGRH-V(Me,C18:1,S)/(Me,C18:1,S)	?	N	+		López-Lara *et al.* (1995b)
	S. rostrata	NodARc factors[g]	10^{-9}	N	+		Mergaert *et al.* (1993)
Root hair deformation (Had), branching, and tip swelling							
	V. sativa	NodRlv-V (Ac,C18:4)	10^{-11}	N	+		Spaink *et al.* (1991)
		NodRlv-IV (Ac,C18:4)	10^{-11}	N	+		
		NodRlv-V (Ac,C18:1)	10^{-11}	N	+		
		NodRlv-IV (Ac,C18:1)	10^{-11}	N	+		
		NodRlv-V (C18:4)	10^{-11}	N	+		
		NodRlv-IV (C18:4)	10^{-11}	N	+		
		NodRlv-V (C18:1)	10^{-11}	N	+		
		NodRlv-IV (C18:1)	10^{-11}	N	+		
		Chitotetraose	?	S	–		
	V. sativa	NodRlv-V (Ac,C18:0)[i]	10^{-10}	N	+	3 h	Heidstra *et al.* (1994)
		NodRlv-III (Ac,C18:4)	10^{-8}	N	+	3 h	de Ruijter *et al.* (1998)
		NodRlv-II (Ac,C18:4)	10^{-7}	N	+	3 h	
	V. sativa	NodSm-IV (C16:2,S)	10^{-6}	N	+		Staehelin *et al.* (1994b)
		NodSm-I V(C16:2)	10^{-9}	N	+		
		NodSm-III (C16:2)	10^{-5}	N[n]	+		
		NodSm-II (C16:2)	10^{-5}	N[n]	+		
	M. sativa	NodSm-IV (Ac,C16:2,S)	10^{-12}	N	+		Roche *et al.* (1991a)
		NodSm-IV (C16:2,S)	10^{-11}	N	+		
		NodSm-IV (Ac,C16:2)	10^{-9}	N	–		
		NodSm-IV (C16:2)	10^{-9}	N	–		
	M. sativa	NodSm-IV (C16:2,S)	10^{-13}	N	+	5 h	Kurkdjian (1995)
	M. sativa	NodSm-IV (C16:2,S)	10^{-10}	N	+		Staehelin *et al.* (1994b)
		NodSm-IV (C16:2)	10^{-6}	N	+		
		NodSm-III (C16:2)	10^{-5}	N[n]	+		
		NodSm-II (C16:2)	10^{-5}	N[n]	+		
	M. sativa	Material 2[o]	10^{-6}	S	+		Staehelin *et al.* (2000)
	L. corniculatus	Nod factor mixture of *M. loti* E1R	10^{-9}	N	+		López-Lara *et al.* (1995a)
	L. japonicus	Nod factor mixture of *M. loti* JRL501	10^{-7}	N	+	6 h [i″]	Niwa *et al.* (2001)
	M. atropurpureum	NodNGR [Ac][h]	10^{-11}	N	+		Relić *et al.* (1993)
		NodNGR [OH]	10^{-11}	N	+		
		NodNGR [S]	10^{-11}	N	+		
	V. unguiculata	NodNGR [Ac,S][j]	10^{-9}	N	+	1 min[k]	Gehring *et al.* (1997)
		NodSm-IV/V [Ac,S][j]	10^{-9}	N	+		
		Chitotetraose	10^{-7}	S	–		
	G. soja	LCO-V (C18:1Δ11,MeFuc)	10^{-15}	S	+[l]		Stokkermans *et al.* (1995)
		LCO-V (C18:1Δ9,MeFuc)	10^{-12}	S	+		
		LCO-V (C16:0,MeFuc)	10^{-10}	S	+		
		LCO-IV (C18:1Δ11,MeFuc)	10^{-8}	S	–		
		LCO-IV (C16:0,MeFuc)	10^{-8}	S	–		
		LCO-IV (C18:1Δ11,Fuc,R-Gro)	10^{-8}	S	–		
		LCO-IV (C18:1Δ11,Fuc,S-Gro)	10^{-8}	S	–		
		LCO-IV (C18:1Δ9,Fuc,R-Gro)	10^{-8}	S	–		
		LCO-IV (C18:1Δ9,Fuc,S-Gro)	10^{-8}	S	–		
		LCO-IV (C16:0)	10^{-14}	S	+		
		LCO-IV (C16:0,S)	10^{-8}	S	–		

Table II. *Continued*

Response	Test plant	Compound[a]	Conc.[b]	N/S[c]	+/–[d]	Time[e]	Reference
		LCO-IV (C16:1Δ2,S)	10^{-8}	S	–		
		LCO-IV (C16:1Δ9,S)	10^{-8}	S	–		
		LCO-IV (C16:2Δ2,9,S)	10^{-8}	S	–		
		NodBe-V (C18:1Δ11,MeFuc)	10^{-8}	N	+		
		NodBj-V (C18:1Δ11)	10^{-8}	N	–		
		NodRlv-V (C18:1Δ11)	10^{-8}	N	–		
	T. repens	NodRlt factors[m]	10^{-9}	N	+[l]		Orgambide *et al.* (1996)
	T. repens	N-C16:0-GlcN	10^{-9}	S	+[l]		Philip-Hollingsworth *et al.* (1997)
		N-C16:2-GlcN	10^{-9}	S	+		
	M. truncatula	NodSm-IV (C16:2,S)	10^{-11}	N	+		Catoira *et al.* (2000)
		NodSm-IV (C16:2)	10^{-7}	N	–		
Induction of root hair curling (Hac)							
	M. atropurpureum	NodNGR [Ac][h,p]	10^{-9}	N	+		Relić *et al.* (1993)
		NodNGR [OH]	10^{-9}	N	+		
		NodNGR [S]	10^{-9}	N	+		
	V. unguiculata	NodNGR [Ac,S]	10^{-9}	N	+	32 min	Gehring *et al.* (1997)
		NodSm-IV/V [Ac,S]	10^{-9}	N	–		
		Chitotetraose	10^{-7}	S	–		
	L. japonicus	Nod factor mixture of *M. loti* JRL501	10^{-7}	N	+		Niwa *et al.* (2001)
Changes in cytoplasmic streaming							
	V. sativa	NodRlv-V (Ac,C18:4)	10^{-9}	N	+	30 min	Heidstra *et al.* (1994)
	P. vulgaris	Nod factors of *R. etli* CE3[g]	10^{-8}	N	+	<10 min	Cárdenas *et al.* (1998)
	M. truncatula	Nod factors of *R. leguminosarum*[g]	10^{-8}	N	+	min	Sieberer and Emons (2000)
Cytoskeleton rearrangements							
	P. vulgaris	Nod factors of *R. etli* CE3[g]	10^{-8}	N	+	10 min	Cárdenas *et al.* (1998)
	V. sativa	NodRlv-V (Ac,C18:4)	10^{-10}	N	+	<1 h	Miller *et al.* (1999)
							de Ruijter *et al.* (1999)
Intracellular alkalinization							
	M. sativa	NodSm-IV (C16:2,S)	10^{-7}	N	+	sec	Felle *et al.* (1996, 2000)
		NodSm-IV (C16:0,S)	10^{-7}	N	+		
		NodSm-V (C16:2,S)	10^{-7}	N	+		
		NodSm-IV (C16:2)	10^{-7}	N	+		
		NodSm-IV (Ac,C16:2,S)	10^{-7}	N	+		
		Chitotriose	10^{-7}	S	–		
		Chitotetraose	10^{-7}	S	+[q]		
		Chitopentaose	10^{-7}	S	+		
		Chitohexaose	10^{-7}	S	–		
		Chitooctaose	10^{-7}	S	+[r]		
Extracellular alkalinization							
	M. sativa	NodSm-IV(C16:2,S)	10^{-9}	N	+	sec	Felle *et al.* (1998)
		Chitooctaose	10^{-8}	S	+	min	Felle *et al.* (2000)
Depolarization of membrane potential							
	M. sativa	NodSm-IV (C16:2,S)	10^{-9}	N	+[s]	10 min	Ehrhardt *et al.* (1992)
	M. sativa	NodSm-IV (C16:2,S)	10^{-8}	N	+	20 min	Felle *et al.* (1995), Kurkdjian (1995)
		NodSm-IV (C16:2)	10^{-7}	N	–		
		NodSm-IV (Ac,C16:2,S)	10^{-8}	N	+		
		Chitotetraose	10^{-6}	S	–		
	M. sativa	NodSm-V (C16:2,S)	10^{-7}	N	+[q]	20 min	Felle *et al.* (1995)
		NodSm-IV (C16:0,S)	10^{-7}	N	+[q]		
	M. sativa	Chitotetraose	10^{-7}	S	–	min	Felle *et al.* (2000)
		Chitohexaose	10^{-7}	S	+[q]		
		Chitoheptaose	10^{-8}	S	+[q]		
		Chitooctaose	10^{-9}	S	+[q]		

Table II. *Continued*

Response	Test plant	Compound[a]	Conc.[b]	N/S[c]	+/–[d]	Time[e]	Reference
Modulation of proton and (calcium) ion fluxes							
	M. sativa	NodSm-IV (Ac,C16:2,S)	≈10^{-8}	N	+[t,u]		Kurkdjian *et al.* (2000)
	M. sativa	NodSm-IV (C16:2,S)	10^{-7}	N	+[u,v]	sec	Felle *et al.* (1998)
		Chitooctaose	10^{-7}	S	+[q]	sec	Felle *et al.* (2000)
	P. vulgaris	Nod factors of *R. etli* CE3[g]	10^{-8}	N	+[u,w]	10 min	Cárdenas *et al.* (1999)
		Chitopentaose	10^{-7}	S	+[q]	10 min	
Changes in Ca^{2+} (spiking,plateau-like increases)							
	V. sativa	NodRlv-V (Ac,C18:4)	10^{-10}	N	+[x,y]	70 min	de Ruijter *et al.* (1998)
	M. sativa	NodSm-IV (Ac,C16:2,S)	10^{-9}	N	+[x,z,a′]	9 min	Ehrhardt *et al.* (1996)
		NodRlv-V (Ac,C18:4)	10^{-9}	N	–		
		Chitotetraose	10^{-9}	S	–		
	M. sativa	NodSm-IV (C16:2,S)	10^{-9}	N	+[u]	sec	Felle *et al.* (1999)
		NodSm-IV (C16:0,S)	10^{-7}	N	+[q]		
		NodSm-IV (Ac,C16:2,S)	10^{-7}	N	+		
		Chitotetraose	10^{-7}	S	–		
	V. unguiculata	NodNGR-V [Ac,S]	10^{-9}	N	+[x,b′]	sec	Gehring *et al.* (1997)
		NodSm-IV/V [Ac,S]	10^{-9}	N	+		
		Chitotetraose	10^{-7}	S	–		
	M. truncatula	NodSm-IV (Ac,C16:2,S)	10^{-9}	N	+[x]	9 min	Wais *et al.* (2000)
	P. vulgaris	Nod factors of *R. etli* CE3[g]	10^{-8}	N	+[x]	sec	Cárdenas *et al.* (1999)
		Chitopentaose	10^{-7}	S	–		
	P. sativum	NodRlv-IV(Ac,C18:4)[c′]	10^{-8}	N	+	min[d′]	Walker *et al.* (2000)
		Chitotetraose	10^{-6}	S	+		
		Chitopentaose	10^{-6}	S	+		
Induction of early nodulin genes							
	P. sativum ENOD12	—[e′]				24 h	Scheres *et al.* (1990)
	P. sativum ENOD12	NodRlv factors[g]	10^{-8}	N	+/+/+[g′]		Horvath *et al.* (1993)
		NodRlv-V (Ac,C18:4)	10^{-8}	N	+/–/–		
		NodRlv-V (Ac,C18:1)	10^{-8}	N	–/+/–		
		NodSm-IV (–/Ac,C16:2,S)[f′]	10^{-8}	N	–/+[q]/+[q]		
	P. sativum ENOD5	NodRlv factors[g]	10^{-8}	N	+/+/+[g′]		Horvath *et al.* (1993)
		NodRlv-V (Ac,C18:4)	10^{-8}	N	+[q]/+/–		
		NodRlv-V (Ac,C18:1)	10^{-8}	N	–/+/–		
		NodSm-IV (–/Ac,C16:2,S)[f′]	10^{-8}	N	–/–/+		
	M. truncatula ENOD12	—[h′]				3–6 h	Pichon *et al.* (1992)
	M. truncatula ENOD12	NodSm-IV (Ac,C16:2,S)	10^{-12}	N	+[i′]	24 h	Journet *et al.* (1994)
		NodSm-IV (Ac,C16:2)	10^{-9}	N	+		Pingret *et al.* (1998)
		NodSm-IV (Ac,C16:0,S)	10^{-11}	N	+		
		NodSm-IV (C16:2,S)	10^{-12}	N	+		
		Chitotetraose	10^{-6}	S	–		
	M. truncatula rip1	NodSm-IV (–/Ac,C16:2,S)[f′]	5 10^{-9}	N	+	6 h	Cook *et al.* (1995)
	V. sativa ENOD5/ENOD12	NodRlv-V (Ac,C18:4)	10^{-10}	N	+	8 h	Vijn *et al.* (1995)
Cortex							
Polar tip growth of outer cortical cells							
	V. sativa	NodRlv factors[g]	10^{-7}	N	+		van Brussel *et al.* (1992)
Formation of pre-infection threads							
	V. sativa	NodRlv factors[g,j′]	10^{-7}	N	+		van Brussel *et al.* (1992)
		NodRlv-IV (Ac,C18:4)	10^{-7}	N	+		
		NodRlv-V (Ac,C18:4)	10^{-7}	N	+		
		NodRlv-IV (Ac,C18:1)	10^{-7}	N	–		
		NodRlv-V (Ac,C18:1)	10^{-7}	N	–		
		NodRlv-IV (C18:4)	10^{-7}	N	–		
		NodRlv-V (C18:4)	10^{-7}	N	–		
	L. japonicus	Nod factor mixture of *M. loti* JRL501	5–10 ng	N	+		Niwa *et al.* (2001)

Table II. *Continued*

Response	Test plant	Compound[a]	Conc.[b]	N/S[c]	+/–[d]	Time[e]	Reference
Formation of nodule primordia (initiated in the inner cortex)							
	V. sativa	NodRlv-V (Ac,C18:4)	5 10^{-8}	N	+		Spaink *et al.* (1991)
		NodRlv-IV (Ac,C18:4)	5 10^{-8}	N	+		
		NodRlv-V (Ac,C18:1)	5 10^{-8}	N	–		
		NodRlv-IV (Ac,C18:1)	5 10^{-8}	N	–		
		NodRlv-V (C18:4)	5 10^{-8}	N	–		
		Chitotetraose		S	–		
	M. sativa	NodSm-IV (Ac,C16:2,S)	10^{-7}	N	+[f,k′,l′]		Roche *et al.* (1991a)
		NodSm-IV (C16:2,S)	10^{-7}	N	+		Truchet *et al.* (1991)
		NodSm-IV (Ac,C16:2)	10^{-7}	N	–		
		NodSm-IV (C16:2)	10^{-7}	N	–		
		NodSm-IV (C16:0,S)	10^{-7}	N	–		
	M. sativa	CO-IV (S,NH_2)	10^{-7}	S	–		Demont-Caulet *et al.* (1999)
		LCO-IV (C18:2Δ2,9,S)	10^{-8}	S	+[q]		
		LCO-IV (C16:2Δ2,9,S)	10^{-8}	S	+		
		LCO-IV (C16:1Δ2,S)	10^{-8}	S	+[q]		
		LCO-IV (C16:1Δ9,S)	10^{-8}	S	+[q]		
		LCO-IV (C8:1Δ2,S)	10^{-8}	S	–		
		LCO-IV (C12:1Δ2,S)	10^{-8}	S	+[q]		
		LCO-IV (C16:1Δ2,S)	10^{-8}	S	+		
		LCO-IV (C18:1Δ2,S)	10^{-8}	S	+[q]		
	T. repens[l]						Orgambide *et al.* (1996), Phillip-Hollingsworth *et al.* (1997)
Formation of nodule primordia (initiated in the outer cortex)							
	L. preslii	NodMl-V (Me,Cb,C18:1,AcFuc)	10^{-4}	N	+		López-Lara *et al.* (1995a)
	P. vulgaris	NodGRH-V (Me,C18:1,S)	10^{-7}	N	+		López-Lara *et al.* (1995b)
		NodGRH-V (Me,C18:1)	10^{-7}	N	+[q]		López-Lara *et al.* (1995b)
	Acacia cyanophylla	NodGRH-V (Me,C18:1,S)	10^{-7}	N	+		
	Acacia melanoxylon	NodGRH-V (Me,C18:1)	10^{-7}	N	+[q]		
	M. atropurpureum	NodNGR [Ac][h,p]	10^{-7}	N	+		Relić *et al.* (1993)
		NodNGR [OH]	10^{-7}	N	+		
		NodNGR [S]	10^{-7}	N	+		
	V. unguiculata	NodNGR [Ac][h,p]	10^{-7}	N	+[m′]		Relić *et al.* (1994)
		NodNGR [OH]	10^{-7}	N	+		
		NodNGR [S]	10^{-7}	N	+		
	G. soja[l,l′]						Stokkermans *et al.* (1995), Stokkermans and Peters (1994)
	V. umbellata	NodBj-V (C18:1Δ11,MeFuc)[p′]	100 ng[o′]	N	+		Cohn *et al.* (1999)
		NodBj-V (C18:1Δ11)	100 ng	N	–		
		LCO-V (C18:1Δ9,MeFuc)	100 ng	S	+		
		LCO-V (C16:0,MeFuc)	100 ng	S	+		
		LCO-V (C16:0)	100 ng	S	–		
		LCO-IV (C16:0)	100 ng	S	–		
		LCO-IV (C18:1Δ11,MeFuc)	100 ng	S	–		
		LCO-IV (C18:1Δ11,MeFuc,R-Gro)	100 ng	S	–		
		LCO-IV (C18:1Δ11,MeFuc,S-Gro)	100 ng	S	–		
		LCO-IV (C16:0,S)	100 ng	S	–		
		LCO-IV (C12:2Δ2,9,S)	100 ng	S	–		
		Mixture 1[q′]	100 ng	N	+		
		Mixture 2[q′]	100 ng	N	+		
		Mixture 3[q′]	100 ng	N	+		
		NodBe-V (Ac,C18:1Δ11,MeFuc)	100 ng	N	+		
		NodBe-V (Ac,Cb,C18:1Δ11,MeFuc)	100 ng	N	+		
		NodBj-V (C16:0)	100 ng	N	–		
		Mixture 4[q′]	100 ng	N	–		

Table II. *Continued*

Response	Test plant	Compound[a]	Conc.[b]	N/S[c]	+/–[d]	Time[e]	Reference
		Mixture 5[q′]	100 ng	N	–		
		Mixture 6[q′]	100 ng	N	–		
		Mixture 7[q′]	100 ng	N	+		
		Mixture 8[q′]	100 ng	N	+		
		Mixture 9[q′]	100 ng	N	+		
	L. corniculatus	NodBe-V (C18:1,MeFuc)	100 ng[o′]	N	+		van Rhijn *et al.* (1998)
		Fraction 2[n′]	100 ng	N	+		
		Fraction 3[n′]	100 ng	N	+		
		NodBj-V (C18:1)	100 ng	N	–		
		LCO-IV (C18:1,Fuc,R-Gro)	100 ng	S	–		
		LCO-IV (C16:0)	100 ng	S	–		
		LCO-IV (C18:1,MeFuc)	100 ng	S	–		
		LCO-IV (C18:1,Fuc,S-Gro)	100 ng	S	–		
	L. japonicus	Nod factor mixture of *M. loti* JRL501	5–10 ng	N	+		Niwa *et al.* (2001)
Local induction of cell cycle genes							
	V. sativa	NodSm-IV (Ac,C16:2,S)	10^{-5}	N	+	20 h	Yang *et al.* (1994)
Induction of early nodulin genes							
	P. sativum ENOD12	—[e′]				2 days	Scheres *et al.* (1990)
	M. truncatula ENOD12	—[h′]				48–72 h	Pichon *et al.* (1992)
	V. sativa ENOD40	NodRlv-V (Ac,C18:4)	10^{-9}	N	+	6 days	Vijn *et al.* (1995)
	M. sativa ENOD12	NodSm-IV (C16:2,S)	10^{-8}	N	+[r′]	4 days	Bauer *et al.* (1996)
	S. rostrata SrGA20OX1	NodARc factors[g]	10^{-8}	N	+	1 h	Lievens (2001)
	S. rostrata Srprx1	NodARc factors[g]	10^{-8}	N	+	30 min	Lievens (2001)
	G. soja ENOD2	LCO-V (C18:1Δ11,MeFuc)	100 ng[o′]	S	–		Minami *et al.* (1996a)
		LCO-V(C16:0,MeFuc)	100 ng	S	–		
		LCO-V (C18:1Δ9,MeFuc)	100 ng	S	–		
		LCO-IV(C16:0)	100 ng	S	–		
		Mixture 1[s′]	100 ng	S	+		
		Mixture 2[s′]	100 ng	S	+		
		Mixture 3[s′]	100 ng	S	+		
		Mixture 4[s′]	100 ng	S	+[q]		
		Mixture 5[s′]	100 ng	S	+		
		NodBj-V (C18:1Δ11)[t′]	100 ng	N	+		
		LCO-IV (C18:1Δ11,MeFuc)[t′]	100 ng	S	+[q]		
		LCO-IV(C16:2Δ2,9,S)[t′]	100 ng	S	+[q]		
		Chitotetraose[t′]	100 ng	S	+		
		Chitopentaose[t′]	100 ng	S	+[q]		
		LCO-IV (C18:1Δ11,MeFuc)[u′]	100 ng	S	+		
		LCO-IV (C16:2Δ2,9,S)[u′]	100 ng	S	+		
	M. truncatula ENOD20	NodSm	10^{-11}	N	+	24 h	Vernoud *et al.* (1999)
		NodSm-IV (Ac,C16:2,S)	10^{-8}	N	+		
		NodSm-IV (Ac,C16:2)	10^{-8}	N	–		
		NodSm-IV (C18:1,S)	10^{-8}	N	+		
		LCO-IV (C16:1,S)	10^{-8}	S	+		
		LCO-IV (C12:1,S)	10^{-8}	S	+		
		LCO-IV (C8:1,S)	10^{-8}	S	+		
		CO-IV (S)	10^{-8}	S	–		
	M. truncatula N6	NodSm-IV (Ac,C16:2,S) [h″]	10^{-7}	N	+	48 h	Mathis *et al.* (1999)
	M. truncatula Ann1	NodRm [j″]	10^{-7}	N	+		de Carvalho Niebel *et al.* (1998)
Pericycle							
Induction of ENOD40							
	V. sativa	NodRlv-V (Ac,C18:4)	10^{-9}	N	+[v′]	6 days	Vijn *et al.* (1995)
	M. sativa	NodSm-IV (C16:2,S)	10^{-9}	N	+	2 days	Crespi *et al.* (1994)
	M. sativa	NodSm-IV (C16:1,S)	10^{-8}	N	+	4 days	Fang and Hirsch (1998)

Table II. *Continued*

Response	Test plant	Compound[a]	Conc.[b]	N/S[c]	+/–[d]	Time[e]	Reference
	G. soja	NodBj-V (C18:1Δ11,MeFuc)	100 ng[o′]	N	+[w′]	12 h	Minami *et al.* (1996b)
		LCO-V (C18:1Δ9,MeFuc)	100 ng	S	+[w′]		
		LCO-V (C16:0,MeFuc)	100 ng	S	+[w′]		
		LCO-IV (C16:0)	100 ng	S	+[w′]		
		LCO-IV (C18:1Δ9,MeFuc)	100 ng	S	+[x′]	40 h	
		LCO-V (C16:2,S)	100 ng	S	+[x′]		
		Chitopentaose	100 ng	S	+[x′]		
Whole root							
Induction of a dimer-forming Nod factor hydrolase							
	M. sativa	NodSm-IV (C16:2,S)	10^{-8}	N	+[y′]		Staehelin *et al*. (1995, 2000)
		NodSm-V (C16:2,S)	10^{-8}	N	+		
		NodSm-IV (Ac,C16:2,S)	10^{-10}	N	+		
		NodSm-V (Ac,C16:2,S)	10^{-8}	N	+		
		NodSm-IV (C16:2)	10^{-6}	N	–		
		NodSm-III (C16:2)	10^{-6}	N	–		
		Chitotetraose	10^{-5}	S	–		
		Chitopentaose	10^{-5}	S	–		
		Material 2[o]	10^{-6}	S	+		
	P. sativum	NodRlv factors[g]	10^{-6}	N	+		Ovtsyna *et al.* (2000)
		NodSm-V (C16:2,S)	10^{-6}	N	+		
		NodRlv-IV (Ac,C18:4)	10^{-10}	N	+		
		NodRlv-IV (Ac,C18:4,Fuc)	10^{-11}	N	+		
		NodRlv-V (Ac,C18:4)	10^{-9}	N	+		
		NodRlv-V (Ac,C18:4,Fuc)	10^{-10}	N	+		
Induction of enzymes with chitinase activity							
	G. max cv. CHI1	NodNGR-V [MeFuc,Ac][g]	10^{-7}	N	+		Xie *et al.* (1999)
		Chitotetraose	10^{-7}	S	+		
Enhancement of mycorrhizal colonization							
	G. max	NodNGR-V [MeFuc,Ac][g]	10^{-9}	N	+		Xie *et al.* (1995)
		NodNGR-V [MeFuc,S][g]	10^{-9}	N	–		
Induction of defense-like responses							
	M. sativa	NodSm-IV (C16:2,S)	10^{-6}	N	+[z′]	30 min	Savouré *et al.* (1997)
		NodSm-IV (C16:2)	10^{-6}	N	–		
		Chitotetraose	10^{-6}	S	–		
Perturbation of auxin transport in cortex and vascular bundles							
	T. repens	NodRlt factors[g]	10^{-8}	N	+[a″]	24 h	Mathesius *et al.* (1998)
		O-acetyl-chitotetraose	10^{-6}	S	+[q]		
		O-acetyl-chitopentaose	10^{-6}	S	+		
		O-acetyl-chitohexaose	10^{-6}	S	+[q]		
		Chitobiose	10^{-6}	S	–		
		Chitotetraose	10^{-6}	S	–		
		Chitopentaose	10^{-6}	S	–		
		Chitohexaose	10^{-6}	S	–		
	V. sativa	NodRlv-IV/V (Ac,C18:4)	10^{-9}	N	+	4 h	Boot *et al.* (1999)
		NodRlv-IV/V (Ac,C18:1)	10^{-7}	N	+		
		NodSm-IV (Ac,C16:2,S)	10^{-7}	N	–		
		Chitopentaose	10^{-6}	S	–		
Expression of MtENOD12 *in transgenic rice plants*							
	Oryza sativa	NodNGR factors[g]	10^{-9}	N	+	24 h	Reddy *et al.* (1998)
		Chitotetraose	10^{-6}	S	–		
Suspension-cultured cells							
Induction of flavonoid synthesis genes							
	M. sativa	NodSm-IV (C16:2,S)	10^{-6}	N	+	2 h	Savouré *et al*. (1994, 1997)
		NodSm-IV (C16:0,S)	10^{-6}	N	+		
		NodSm-IV (C16:2)	10^{-6}	N	–		

Table II. *Continued*

Response	Test plant	Compound[a]	Conc.[b]	N/S[c]	+/–[d]	Time[e]	Reference
Induction of cell cycle genes							
	M. sativa	NodSm-IV (C16:2,S)	10^{-9}	N	+	2 h	Savouré *et al.* (1994)
		NodSm-IV (C16:0,S)	10^{-6}	N	+		
		NodSm-IV (C16:2)	10^{-6}	N	–		
Complementation of an embryogenesis mutant							
	D. carota	NodRlv-V (Ac,C18:4)	10^{-9}	N	+		De Jong *et al.* (1993)
		NodRlv-V(Ac,C18:1)	10^{-8}	N	+		
		Chitopentaose	10^{-7}	S	–		
Alkalinization							
	M. sativa	NodSm factors[g]	10^{-5}	N	–[b″]	5 min	Baier *et al.* (1999)
		Chitotriose	10^{-4}	S	–		
		Chitotetraose	10^{-4}	S	+		
		Chitopentaose	10^{-6}	S	+		
		Chitohexaose	10^{-4}	S	+		
	N. tabacum	NodSm factors[g]	10^{-5}	N	+	5 min	Baier *et al.* (1999)
		Chitotriose	10^{-4}	S	–		
		Chitotetraose	10^{-4}	S	+		
		Chitopentaose	10^{-6}	S	+		
		Chitohexaose	10^{-4}	S	+		
	L. esculentum	NodNGR [MeFuc][g]	10^{-9}	N	+		Staehelin *et al.* (1994a)
		NodNGR [MeFuc,S][g]	10^{-9}	N	+		
		NodNGR [MeFuc,Ac][g]	10^{-9}	N	+		
		Chitotetraose	10^{-9}	S	+		
		NodNGR factors[g]	10^{-9}	N	+		
		NodRlv factors[g]	10^{-9}	N	+		
		NodSm factors[g]	10^{-9}	N	+		
		NodRe factors[g]	10^{-9}	N	+		
		NodBj factors[g]	10^{-9}	N	+		
Hydrogen peroxide production							
	M. sativa	NodSm factors[g]	10^{-5}	N	–		Baier *et al.* (1999)
		Chitopentaose	10^{-5}	S	–		
	N. tabacum	NodSm factors[g]	10^{-5}	N	+	10 min	Baier *et al.* (1999)
		Chitopentaose	10^{-5}	S	+		
Ca^{2+} influx							
	G. max	NodBj-V (C18:1,MeFuc)	10^{-9}	N	+[x,c″]	<1 min	Yokoyama *et al.* (2000)
	G. soja	NodBj-V (C18:1)	10^{-8}	N	–		
	G. max[d″]	NodNGR-V [MeFuc,Ac]	10^{-7}	N	+[q]	min	Müller *et al.* (2000)
		NodNGR-V [MeFuc,S]	10^{-7}	N	–[e″]		
		NodNGR factors	10^{-7}	N	+		
		NodRt-V (Me,C18:1)	10^{-7}	N	+		
		NodRt-V (Me,C18:1,S)	10^{-7}	N	+		
		NodSm-IV (C16:2,S)	10^{-7}	N	+		
		NodSm-IV (Ac,C16:2,S)	10^{-7}	N	+[q]		
		NodSm-V (C16:2,S)	10^{-7}	N	+		
		NodSm-III (C16:2)	10^{-7}	N	–[e″]		
		NodSm-II (C16:2)	10^{-7}	N	–[e″]		
		Chitotetraose	10^{-7}	S	+[f″]		
		Chitopentaose	10^{-7}	S	+[f″]		
Protoplast cultures							
Ca^{2+} influx							
	G. max	NodBj-V (C18:1,MeFuc)	10^{-9}	N	+[x,g″]	<1 min	Yokoyama *et al.* (2000)
		NodBj-V (C18:1)	10^{-8}	N	–		
Protoplast division and regeneration of pro-embryogenic masses							
	Picea abies	NodNGR factors[g]	10^{-8}	N	+		Dyachok *et al.* (2000)

Table II. *Continued*

[a]Nomenclature as described in Table I.
[b]The concentration in molar, unless otherwise stated, to obtain a significant response.
[c]Indication whether the compound used in the respective assay is natural (N) or synthetic (S).
[d]Indication of a response (+) or no response (–).
[e]Indication of the time at which the response was first observed. If no time indication is mentioned, it was not clear when the response could be first observed, or the effect was not followed in time.
[f]Demont-Caulet *et al.* (1999) used synthetic Nod factor analogs and reached results similar to those of Roche *et al.* (1991a).
[g]A mixture of Nod factors was applied.
[h]Mixtures of NodNGR factors, carrying on the MeFuc group at the reducing-terminal residue an acetyl or hydroxyl group, or a sulfate ester, respectively.
[i]The Nod factors tested by Spaink *et al.* (1991) were also used by Heidstra *et al.* (1994).
[j]Supposedly, Nod factor mixtures were used.
[k]Root hair deformation was scored in time: as early as 1 min after Nod factors were added to roots, bulges with outgrowths could be detected; at 2 min, branching was observed; at 4 min, twisted root hairs were present; and, at 32 min, curled root hairs could be seen.
[l]All Nod factors or synthetic analogs that induced root hair deformations, also induced cortical cell divisions.
[m]Mixture of Nod factors isolated from *R. leguminosarum* bv. *trifolii*.
[n]NodSm-III (C16:2) and NodSm-II (C16:2) are degradation products of NodSm-IV (C16:2,S).
[o]Material 2 contained a mixture of GlcN(C16:2)-GlcNAc-GlcNH$_2$-GlcNAc(OH)(S), GlcN(C16:2)-GlcNAc-GlcNH$_2$-GlcNH$_2$(OH)(S), GlcN(C16:2)-GlcNH$_2$-GlcNH$_2$-GlcNH$_2$(OH)(S), GlcN(C16:2)-GlcNAc-GlcNAc-GlcNH$_2$(OH)(S), and GlcN(C16:2)-GlcNH$_2$-GlcNH$_2$-GlcNAc(OH)(S), derived from an enzymatic *N*-deacetylation of NodSm-IV (C16:2,S).
[p]NodBj-V (C18:1), NodRlv-IV (Ac,C18:4), and NodSm-IV (Ac,C16:2,S) also induced root hair curling, but less efficiently.
[q]Weak response compared to (other) Nod factors or (other) chitooligosaccharides, dependent on what is applicable.
[r]Weak alkalinization followed by acidification.
[s]No response was observed on tomato.
[t]Conclusion based on the effect of Nod factors on membrane potential changes in the presence of La^{3+} or Mg^{2+}.
[u]Measured by using electrodes.
[v]Ca^{2+} influx, Cl^- and H^+ efflux.
[w]Ca^{2+} influx at the root hair apex.
[x]Measured with sensitive dyes.
[y]An increase in [Ca^{2+}] was observed in the root hair tip.
[z]Ca^{2+} spiking was noticed 9 min after application of Nod factors.
[a′]NodSm-IV (C16:2,S) did not induce [Ca^{2+}] spiking on *V. hirsuta* root hairs, but NodRlv-V (Ac,C18:4) did.
[b′]Within seconds a plateau-like increase in [Ca^{2+}] was reached. No such response could be detected when *A. thaliana*, *Petrosolinum crispum*, or *Zea mays* were treated with Nod factors or chitotetraose.
[c′]Contaminated with approximately 10% NodRlv-V (Ac,C18:1).
[d′]One to 2 min after application of Nod factors an increase in Ca^{2+} was observed, followed by [Ca^{2+}] spiking that started approximately 5–15 min later.
[e′]Expression of *PsENOD12* was shown to be *nodABC*/*nodE* dependent.
[f′]A mixture of NodSm-IV (C16:2,S) and NodSm-IV (Ac,C16:2,S).
[g′]Induction of the particular early nodulin gene is indicated, 12/24/48 h after application of Nod factors.
[h′]Expression of *MtENOD12* was shown to be *nodA*-dependent.
[i′]Expression studied in *M. varia* transgenic plants carrying a *MtENOD12-gus* fusion.
[j′]The mixture consisted of NodRlv-IV (Ac,C18:4), NodRlv-V (Ac,C18:4), NodRlv-IV (Ac,C18:1), and NodRlv-V (Ac,C18:1).
[k′]A mixture of NodSm-IV (C16:2,S) and NodSm-IV (Ac,C16:2,S) (60/40) gave a stronger response than when each of the Nod factors was added separately.
[l′]These nodule primordia developed into complete nodules.
[m′]A similar response was observed on *Calopogonium caeruleum* and *G. max*.
[n′]Fraction 2: NodBe-V (Ac,C18:1,MeFuc) and NodBe-IV (C18:1,Fuc,Gro); Fraction 3: NodBe-IV (C18:1,MeFuc), NodBe-IV (Cb,C18:1,MeFuc), NodBe-IV (Cb,C18:1,Fuc,Gro), NodBe-IV (Me,Cb,C18:1,MeFuc), NodBe-IV (Me,C18:1,MeFuc), and NodBe-IV (Me,Cb,C18:1,Fuc,Gro).
[o′]ng of each molecule was applied per spot inoculation.
[p′]NodBe-V (C18:1Δ11,MeFuc) and LCO-V (C18:1Δ11,MeFuc) induced similar respones.
[q′]Mixture 1: NodBe-V (Me,C18:1Δ11,Fuc,Gro) and NodBe-V (Me,C16:0,Fuc,Gro); mixture 2: NodBe-V (Ac,C18:1Δ11,MeFuc) and NodBe-IV (C18:1Δ11,Fuc,Gro); mixture 3: NodBe-IV (C18:1Δ11,MeFuc), NodBe-IV (Cb,C18:1Δ11,Fuc,Gro), NodBe-V (Me,Cb,C18:1Δ11,MeFuc), NodBe-IV (Me,C18:1Δ11,MeFuc) and NodBe-IV (Me,Cb,C18:1Δ11,Fuc,Gro); mixture 4: NodBj-IV (C18:1Δ11,Gro) and NodBj-V (C18:1Δ11); mixture 5: NodBj-IV (C18:1Δ11) and NodBj-V (Ac,C18:1Δ11); mixture 6: NodBj-V (Ac,C18:1Δ11) and NodBj-V (C18:1Δ11); mixture 7: NodBj-IV (C18:1Δ11,Gro), NodBj-IV (C18:1Δ11), NodBj-V (Ac,C18:1Δ11) and NodBj-V (C18:1Δ11); mixture 8: NodBj-V (C18:1Δ11), NodBj-IV (C18:1Δ11,Gro) and NodBj-V (C18:1Δ11); mixture 9: NodBj-V (C18:1Δ11), NodBe-IV (C18:1Δ11) and NodBj-V (Ac,C18:1Δ11).
[r′]Induced in dividing cells of the Nod factor–induced nodule primordia.
[s′]Mixture 1 contained all four LCOs mentioned. Mixture 2: mixture 1 lacking LCO-V (C18:1Δ9,MeFuc); mixture 3: LCO-V (C18:1Δ11,MeFuc) and LCO-V (C16:0,MeFuc); mixture 4: LCO-V (C18:1Δ11,MeFuc) and LCO-IV (C16:0); mixture 5: LCO-V (C16:0,MeFuc) and LCO-IV (C16:0).
[t′]In coinoculation with LCO-V (C18:1Δ11,MeFuc).
[u′]In coinoculation with LCO-V (C16:0,MeFuc).
[v′]Also expressed in Nod factor–induced nodule primordia.
[w′]Expression was induced 12 h after Nod factor treatment, abundant at 40 h, and at 6 days a second rise could be observed.
[x′]Expression was abundant at 40 h but decreased to background levels at 6 days. This pattern of expression was induced by molecules that could neither induce root hair deformation nor cortical cell division on *G. soja*.
[y′]Kinetics of the dimer-forming hydrolase were determined using NodSm-IV (C16:2,S) as a substrate.
[z′]The expression of a chalcone synthase and isoflavone reductase, but not that of pathogenesis-related gene and peroxidase gene, was induced.
[a″]Influence of Nod factors and other compounds was tested using transgenic *T. repens* plants carrying a *GH3-gus* contstruct. *GH3* is an auxin-responsive promoter. The Nod factor–induced response consisted of a local and transient down-regulation, followed by a basipetal up-regulation.
[b″]No alkalinization, but rather a slight acidification was observed.
[c″]No response was detected for *T. pratense*.
[d″]Changes in [Ca^{2+}] were measured by aequorin (transgenic *G. max* plants).
[e″]Very weak response.
[f″]Very strong response.
[g″]A weak decrease in cytoplasmic [Ca^{2+}] was observed on *N. tabacum*.
[h″]*S. meliloti nodH*, and *nodFL* mutants, producing Nod factors without sulfate ester at the reducing-terminal residue, and with an altered acyl chain and without acetyl group at the nonreducing-terminal residue, respectively, were unable to induce *MtN6* expression (Mathis *et al.*, 1999).
[i″]Root hair tip swelling was observed as early as 6 h after inoculation with a *M. loti* JRL501 Nod factor mixture.
[j″]An *S. meliloti nodH* did not induce *MtAnn1* expression in contrast to a *nodFL* mutant (de Carvalho Niebel *et al.*, 1998).

Nod factor–induced extracellular alkalinization (Felle *et al.*, 2000). A preincubation of roots with chitooctaose reduces the response induced by NodSm-IV (C16:2,S), but simultaneous addition has no effect (Felle *et al.*, 2000).

Depolarization of the membrane. Ehrhardt *et al.* (1992) were the first to demonstrate that 10^{-9} M NodSm-IV (C16:2,S) applied to alfalfa root hairs depolarizes the membrane within 10 min. NodSm-IV (C16:2,S) and NodSm-IV (Ac,C16:2,S) are equally active and more active than NodSm-V (C16:2,S) and NodSm-IV (C16:0,S). NodSm-IV (C16:2) and chitotetraose cannot induce an important depolarization of the membrane potential (Felle *et al.*, 1995; Kurkdjian, 1995) (Table II). At a concentration of 10^{-7} M, chitotetraose does not trigger this response, in contrast to chitohexaose, -heptaose, and -octaose that weakly depolarize the membrane (Felle *et al.*, 2000) (Table II).

Ion fluxes. The Nod factor–induced depolarization of the membrane potential and extracellular alkalinization of the alfalfa root surface are inhibited on deactivation of the plasma membrane H^+ ATPases, suggesting a role for proton pumps (Felle *et al.*, 1998). A rapid increase in $[Cl^-]$ is seen at the root surface when 10^{-7} M NodSm-IV (C16:2,S) is added to alfalfa roots, whereas, concomitantly, $[Cl^-]$ decreases within the root hairs (Felle *et al.*, 1998). This response occurs after $[Ca^{2+}]$ changes but simultaneously with depolarization of the membrane potential and extracellular alkalinization (Felle *et al.*, 1998). Slightly later than the Cl^- efflux, a transient increase of $[K^+]$ is noticed in the root hair zone (Felle *et al.*, 1998). Chitooctaose induces similar ion fluxes, although less pronounced (Felle *et al.*, 2000). By using a single-electrode voltage-clamp technique, the involvement of anion and $[K^+]$ channel currents together with those of H^+ pumps in Nod factor–induced electrical responses was confirmed (Kurkdjian *et al.*, 2000).

Changes in $[Ca^{2+}]$. Rapid changes in cytosolic $[Ca^{2+}]$, which occur in different patterns, have been documented in a variety of systems (Table II). Approximately 9 min after Nod factors of *S. meliloti* are added to alfalfa root hairs, regular oscillations in $[Ca^{2+}]$ are induced, referred to as spiking, that last for 20–60 min (Ehrhardt *et al.*, 1996). The response is specific because NodSm-IV (Ac,C16:2,S) induces $[Ca^{2+}]$ spiking, in contrast to chitotetraose or NodRlv-V (Ac,C18:4), whereas the latter Nod factor induces $[Ca^{2+}]$ spiking on *Vicia hirsuta*. No significant response can be monitored when *S. meliloti* Nod factors are added to *Lycopersicon esculentum* (tomato) root hairs (Ehrhardt *et al.*, 1996). $[Ca^{2+}]$ spiking is also observed in *M. truncatula* (Wais *et al.*, 2000) and *Pisum sativum* (pea), in which 10^{-9} M NodRlv-V (Ac,C18:4) first induces an increase in $[Ca^{2+}]$, 1–2 min after Nod factor addition, followed 5–15 min later by $[Ca^{2+}]$ spiking that lasts for 40–60 min (Walker *et al.*, 2000). Surprisingly, 10^{-6} M chitotetraose and -pentaose induce $[Ca^{2+}]$ spiking in pea root hairs, similarly to Nod factors (Walker *et al.*, 2000). Recently, mutants of *M. truncatula* (Catoira *et al.*, 2000) and pea (Walker *et al.*, 2000) mutants, affected in initial steps of nodulation (e.g., root hair responses and induction of early nodulins), have been analyzed for their ability to exhibit Nod factor–induced $[Ca^{2+}]$ spiking. This analysis allowed particular plant genes to be positioned in the cascade of Nod factor perception–$[Ca^{2+}]$ spiking–root hair deformation–expression of early nodulins (Wais *et al.*, 2000; Walker *et al.*, 2000).

In other systems, more general increases in $[Ca^{2+}]$ have been observed (Table II). A plateau-like increase in $[Ca^{2+}]$ is induced by 10^{-9} M NodNGR [Ac,S] or NodSm-IV,V [Ac,S] on *Vigna unguiculata* within seconds (Gehring *et al.*, 1997). Such a response is not observed when *V. unguiculata* and *Arabidopsis thaliana* roots are treated with chitotetraose and with Nod factors or chitin fragments, respectively (Gehring *et al.*, 1997). In *V. unguiculata* root hairs, Ca^{2+} localization reveals that within 2 min after Nod factor application, $[Ca^{2+}]$ increased at the very tip of the root hair and in some spots throughout the root hair (Gehring *et al.*, 1997). In alfalfa root hairs, an extracellular decrease in $[Ca^{2+}]$ is measured in the responsive root zone after application of NodSm-IV (C16:2,S) (Felle *et al.*, 1998). The Nod factor–induced Ca^{2+} influx precedes the depolarization of the membrane potential and the transient extracellular alkalinization by several s. The Ca^{2+} influx is required for triggering these events. Ca^{2+} channel antagonists prevent both membrane depolarization and pH changes (Felle *et al.*, 1998). On Nod factor treatment of root hairs of *Phaseolus vulgaris* (common bean), cytosolic $[Ca^{2+}]$ increases in the root hair tip and in spots near the nucleus, often spreading all over the cytoplasm (Cárdenas *et al.*, 1999). These responses occur within seconds; 10–15 min later, fluctuations and changes in $[Ca^{2+}]$ in the nuclear region are observed. Chitin fragments fail to change the cytosolic Ca^{2+} (Cárdenas *et al.*, 1999).

Striking similarities in $[Ca^{2+}]$ fluctuations between growing root hairs and pollen tubes have been reported. In growing pollen tubes of *Lilium*, the apical gradient of $[Ca^{2+}]$ is steep and is required for pollen tube growth (Miller *et al.*, 1992). Also, root hair growth has been correlated with an increased cytosolic $[Ca^{2+}]$ in the apex (de Ruijter *et al.*, 1998). Miller *et al.* (1992) proposed that the region of elevated $[Ca^{2+}]$ may create conditions favoring vesicle fusion. Within the cytosol of living cells, increased $[Ca^{2+}]$ activates arrays of both rapid and sustained responses. Oscillations and their frequencies may have both quantitative and qualitative influences on gene expression (Meldolesi, 1998). Consequently, changes in $[Ca^{2+}]$—either rapid plateau-like increases, slightly later occurring $[Ca^{2+}]$ spiking, or both—may activate a set of genes involved in triggering the onset of nodule development.

Effects on root hair formation or shape. Depending on the symbiotic system, Nod factors can induce the formation of root hairs (Hai) and/or deform existing ones (Had) (e.g., Roche *et al.*, 1991a; Spaink *et al.*, 1991; López-Lara *et al.*, 1995a) (Table II). Only in *Macroptilium atropurpureum* (Relić *et al.*, 1993), *V. unguiculata* (Gehring *et al.*, 1997), and *L. japonicus* (Niwa *et al.*, 2001) Nod factors are sufficient to induce the formation of the typical shepherd's crooks (Table II). Root hairs of *V. unguiculata* treated with NodNGR factors deform rapidly. Within 1 min, a bulge appears that carries an initiation of a root hair branch; at 2 min, branched root hairs are formed; at 4 min, root hairs are deformed and twisted; and at 32 min, root hairs with a shepherd's crook are apparent (Gehring *et al.*, 1997). Nod factor–induced root hair deformation has been followed also in time on *Vicia sativa* (Heidstra *et al.*, 1994). Root hairs of the susceptible zone respond to 10^{-9} M NodRlv-V (Ac,C18:4), and at 30 min an increased cytoplasmic streaming is observed; at 1 h, root hair tips start to swell; at 2 h, polar

growth is initiated; and at 3 h, 80% of the root hairs in zone II is deformed (Heidstra *et al.*, 1994). Strikingly, Nod factor–induced root hair deformation on *V. sativa* takes much longer than on *V. unguiculata* and requires *de novo* protein synthesis.

Root hair cytoskeleton rearrangements. Control root hairs or root hairs treated with 10^{-7} M chitopentaose show long actin bundles running along the root hair from the tip to the base (Cárdenas *et al.*, 1998). After 10^{-8} M Nod factors of *R. etli* have been applied for 5–10 min, a breakdown of microfilament bundles is observed. Approximately 1 h later, the structure of the cytoskeleton is partially recovered, but the actin microfilament stain still accumulates in the root hair tip. These cytoskeleton rearrangements may be a prerequisite for root hair deformation (Cárdenas *et al.*, 1998).

An extensive study of Nod factor–induced cytoskeleton rearrangements has been performed on root hairs of *V. sativa* (de Ruijter *et al.*, 1998). Zone I root hairs exhibited a polar organization of the cytoplasm. In a clear zone at the tip, the cytoplasm contains almost exclusively Golgi vesicles. The subapical region is rich in organelles (endoplasmic reticulum, Golgi apparatus, mitochondria, plastids) and has small vacuoles. Zone II root hairs also have a cytoplasmic polarity and contain large organelles, including vacuoles, up to the tip. Zone III root hairs lack cytoplasmic polarity, and the large vacuoles, which occupy nearly the complete root hair cell, are surrounded by only a tiny layer of cytoplasm (de Ruijter *et al.*, 1998). Spectrin, a large multifunctional protein, that has actin- and calmodulin-binding sites and is part of the membrane-associated cytoskeleton, has been used as a molecular marker. Spectrin accumulates strongly in the cytoplasm of the apex of zone I, only very weakly in zone II, and not at all in zone III root hairs. After 10^{-10} M NodRlv-V (Ac,C18:4) is added to *V. sativa* roots, zone II root hair tips start to swell, a small clear zone at the tip is formed from which a new tip emerges. After Nod factor treatment for 1–2 h, spectrin accumulates and is found at the plasma membrane of the tip swelling, suggesting that Nod factors reinitiate tip growth with features comparable to those of growing root hairs in zone I (de Ruijter *et al.*, 1998). Zone I root hairs have an increased cytosolic [Ca^{2+}] in the tip region; 70 min after addition of Nod factors, a high cytoplasmic [Ca^{2+}] is observed at the plasma membrane of the swelling zone II root hair tips and at the reinitiated tip, which originates from the swelling and forms a new root hair on an existing one (de Ruijter *et al.*, 1998).

During root hair development, one of the first visible events is the formation of a swelling, called a bulge, on an epidermal cell (Miller *et al.*, 1999). These bulges develop into a growing root hair, the apex of which exclusively contains a vesicle-rich zone. Actin filament bundles within growing root hairs are oriented longitudinally, and perpendicularly to those in the epidermal cell. The subapical part exposes fine bundles of actin (Miller *et al.*, 1999). Actin filaments in Nod factor–induced bulges are positioned close to the plasma membrane in various orientations. At that point, the root hair cell changes its growth from unidirectional, as bulge, to polar growth, thereby initiating root hair deformation (Miller *et al.*, 1999). Thus, Nod factors reinitiate elongation of fine-bundle actin and vesicle delivery to the apical region that is free of actin filament bundles. Indeed, cytochalasin D (a compound that binds to the distal part of growing actin filaments, thereby blocking filament elongation and tip growth) blocks Nod factor–induced reinitiation of root hair tip growth but not bulge formation (Miller *et al.*, 1999).

Cortex and pericycle

Induction of early nodulins. Nod factors induce several plant genes that are expressed during early stages of nodulation, the so-called early nodulin or *ENOD* genes. *PsENOD12*, encoding a hydroxyproline-rich protein, is first expressed in root hairs, 24 h after inoculation. Two days after inoculation, transcripts are detected in root hairs, in cortical cells that contain an infection thread, in cells that are ready for infection thread passage, and in inner cortical cells that will form the nodule primordium (Scheres *et al.*, 1990). *PsENOD12* expression is induced transiently after 10^{-8} M of Nod factors from *R. leguminosarum* bv. *viciae*, have been applied for 12, 24, and 48 h (Horvath *et al.*, 1993) (Table II). For *MtENOD12* expression to be induced by *S. meliloti* Nod factors, final concentrations of 10^{-12} M for NodSm-IV (Ac,C16:2,S) and NodSm-IV (C16:2,S), 10^{-11} M for NodSm-IV (Ac,C16:0,S), and 10^{-9} M for NodSm-IV (Ac,C16:2) are required to obtain comparable responses, suggesting that both the presence of a C16:2 fatty acid and a sulfate ester are important. Chitotetraose, even at a concentration of 10^{-6} M, does not induce *MtENOD12* expression (Journet *et al.*, 1994). The *Rhizobium*-induced peroxidase gene (*rip1*) of *M. truncatula*, in which the *in situ* transcript localization coincides with early infection events and initiation of nodule development, is induced by a mixture of NodSm-IV (Ac,C16:2,S) and NodSm-IV (C16:2,S) at a final concentration of approximately 10^{-9} M (Cook *et al.*, 1995) (Table II).

Another interesting case is *ENOD2* of *Glycine soja*, which is expressed in nodule parenchyma (Minami *et al.*, 1996a) but not when roots are treated with either LCO-V (C18:1Δ11, MeFuc), LCO-V (C16:0,MeFuc), LCO-V (C18:1Δ9,MeFuc), or LCO-IV (C16:0); however, *GsENOD2* expression is induced by particular combinations of these Nod factors (Table II), and *GsENOD2* transcripts can be detected only in the parenchyma of nodule structures induced by a Nod factor mixture. Thus such a mixture may be required for nodule ontogeny to progress, which may explain why rhizobia produce populations of structurally different Nod factors (Table II) (Minami *et al.*, 1996a). More examples of *ENOD* genes that are Nod factor–induced are listed in Table II.

Cytoplasmic bridges. Cytoplasmic bridges are observed in outer cortical cells of *V. sativa* (van Brussel *et al.*, 1992) and pea (Bakhuizen, 1988), are positioned in line with young radial walls in the inner cortex, and form preinfection threads through which infection threads grow. Mitogenic Nod factors, which induce nodule primordia formation in *V. sativa* at a final concentration of 10^{-7} M, are sufficient to form preinfection threads (van Brussel *et al.*, 1992) (Table II). Interestingly, Niwa *et al.* (2001) observed that *M. loti* JRL501 Nod factors induced preinfection thread formation in outer cortical cells of *L. japonicus*. Yang *et al.* (1994) showed that outer cortical cells of pea entered the cell cycle but were arrested in G2 to form preinfection threads, whereas inner cortical cells went through the complete cell cycle, leading to nodule primordium formation.

Nodule primordium formation. In many systems, Nod factors induce local foci of cell divisions, forming nodule primordia that develop into nodular structures (Table II). In *M. sativa*, 10^{-7} M NodSm-IV (Ac,C16:2,S) and NodSm-IV (C16:2,S) but not NodSm-IV (Ac,C16:2), NodSm-IV (C16:2), or NodSm-IV (C16:0,S) form discrete foci of cell division, which develop into structures that are microscopically comparable to genuine nodules (Truchet *et al.*, 1991). A mixture of NodSm-IV (Ac,C16:2,S) and NodSm-IV (C16:2,S) (40/60) give a stronger response than each Nod factor separately. Demont-Caulet *et al.* (1999) illustrated how important the correct fatty acid is for induction of cortical cell divisions. A chitotetramer carrying a sulfate ester at the reducing-terminal residue does not trigger any response on alfalfa roots. The length as well as the number and positions of the double bonds of the fatty acids are important for efficient formation of nodule primordia (Table II). Maybe one particular fatty acid at the nonreducing-terminal residue of the Nod factor serves as hydrophobic tail that allows insertion in lipid bilayers, such as the plant plasma membrane (Demont-Caulet *et al.*, 1999). However, foci of cell division can be induced when chitopentaose, substituted with an *O*-acetyl group at the nonreducing-terminal residue is delivered, together with uridine, to cortical cells of *V. sativa* by ballistic microtargeting. This response is observed neither when chitopentaose is used as projectile, nor when O-acetylated chitopentaose is applied externally to roots of *V. sativa* (Schlaman *et al.*, 1997).

A mixture of 10^{-5} M Nod factors, produced by *B. elkanii* USDA61, induces complete nodule structures on spot-inoculated roots of *G. soja* (Stokkermans and Peters, 1994). An extensive structure–function study has been performed on *G. soja* with natural and synthetic *Bradyrhizobium* Nod factors and derivatives (Stokkermans *et al.*, 1995) (Table II). All compounds that cause root hair deformation elicit also nodule primordium formation. The methyl fucosyl group at the reducing-terminal residue is required for biological activity, but the place of the double bond in the fatty acid is not critical. When a pentameric LCO is replaced by a tetramer while retaining all the other substitutions, activity is lost (Table II). However, LCO-IV (C16:0) can induce root hair deformation and nodule primordium formation, suggesting that the MeFuc group at the reducing-terminal residue, required for activity of a pentameric Nod factors, hinders activity when present at the reducing-terminal residue of a tetrameric Nod factor (Stokkermans *et al.*, 1995).

More general Nod factor–induced responses. Colonization of *G. max* roots by the mycorrhizal fungus *Glomus mosseae* is stimulated by 10^{-9} M NodNGR-V [MeFuc,Ac], but not by NodNGR-V [MeFuc,S] (Xie *et al.*, 1995). Mathesius *et al.* (1998) constructed transgenic *Trifolium repens* (white clover) plants that carried an auxin-responsive promoter, GH3, fused to a β-glucuronidase (*gusA*) reporter gene, allowing changes to be assessed in auxin balances during early stages of nodule development. Spot inoculation with 10^{-8} M Nod factors of *R. leguminosarum* bv. *trifolii* results in a local and acropetally transient down-regulation and basipetal up-regulation of auxin transport in the cortex and vascular bundles 24 h after Nod factors have been added. This response is similar to that caused by naphthylphthalamic acid, a common auxin transport inhibitor. O-acetylated chitopentaose and, to a lesser extent, O-acetylated chitotetraose and chitohexaose (but not chitin oligomers) can mimic this response, suggesting that during early stages one of the effects of Nod factors may be the perturbation of auxin transport (Mathesius *et al.*, 1998).

Suspension-cultured cells of *M. sativa* and *Nicotiana tabacum* (tobacco) have been used to demonstrate differences between chitooligomers and Nod factors in induction of extracellular alkalinization and release of hydrogen peroxide (Table II). Chitotrimers, chitotetramers, chitopentamers, and chitohexamers induce an increase of the extracellular pH of *M. sativa* and *N. tabacum* suspension-cultured cells, at a concentration of 10^{-4}, 10^{-4}, 10^{-6}, and 10^{-4} M, respectively, and within 5 min. Nod factors of *S. meliloti*, however, induce alkalinization only in *N. tabacum* suspension-cultured cells, 30 min after Nod factor application. Both 10^{-5} M NodSm factors and chitopentaose increase the production of hydrogen peroxide in *N. tabacum* but not in *M. sativa* suspension-cultured cells (Baier *et al.*, 1999) (Table II).

Nod factor processing

The chitin backbone of Nod factors can be hydrolyzed by chitinases. Table III gives an overview of leguminous and nonleguminous chitinases and other enzymes that degrade Nod factors. Plant chitinases can be components of plant defense reactions, have an antifungal activity, play a role in carrot somatic embryo development, and be involved in plant development (see Collinge *et al.*, 1993; Brunner *et al.*, 1998). A growing body of evidence suggests that chitinases may also play a role during nodule development. Chitinases are present in the cortex of soybean nodules, induced by *B. japonicum* 61-A-101, supposedly to protect central tissues against pathogen invasion (Staehelin *et al.*, 1992); Chinese *G. max* cultivars produce several chitinase isoforms during nodule development (Xie *et al.*, 1999); during the *S. meliloti–M. sativa* symbiosis, chitinases have been detected in necrotic cells in the cortex, involved in infection thread abortion (Vasse *et al.*, 1993); and, during stem nodule development on *S. rostrata*, *Srchi13*, an early nodulin gene, encoding class III chitinase is expressed around infection pockets, around the developing nodule, and in uninfected cells of the central tissue (Goormachtig *et al.*, 1998). Recently, eight different chitinase genes from *M. truncatula* have been isolated and characterized, some of which are expressed during *S. meliloti*–induced nodule development. The expression patterns during nodule development differ from those generated on mycorrhiza or pathogen infection (Salzer *et al.*, 2000).

Distinct isoforms of chitinases and related enzymes cleave specific β-1,4 linkages within the Nod factor chitooligosaccharide backbone, yielding di-, tri-, or tetrameric acylated degradation products (Table III). Leguminous or nonleguminous chitinases belonging to the same class have somehow similar substrate specificities and degrading activities (Table III). An exception is the class I chitinase isolated from *V. unguiculata* that can degrade all NodNGR factors, in contrast to class I chitinases from *P. vulgaris* and *P. sativum*, that use NodNGR-V (MeFuc) but not NodNGR-V (MeFuc,Ac) and NodNGR-V (MeFuc,S) (Staehelin *et al.*, 1994a). The Nod factor structure—including modifications at the nonreducing- and reducing-terminal residues, the fatty acyl chain, the oligomerization degree, and the N-acetylations—may contribute to Nod factor stability against degradation (Table III). The presence of a sulfate ester

Table III. Nod factor–degrading enzymes and activities[a]

Enzyme type[b]	Plant origin	Substrates[c]	Degradation	Acylated reaction products	Reference
Chitinase class I[d]					
	V. unguiculata	NodNGR-V (MeFuc)[e]	+[f,g]	Unknown	Staehelin *et al.* (1994a)
		NodNGR-V (MeFuc,S)[e]	+[g]	Unknown	
		NodNGR-V (MeFuc,Ac)[e]	+[g]	Unknown	
	P. vulgaris	NodNGR-V (MeFuc)	+[g]	Unknown	Staehelin *et al.* (1994a)
		NodNGR-V (MeFuc,S)	–[g]	Unknown	
		NodNGR-V (MeFuc,Ac)	–[g]	Unknown	
	P. sativum	NodNGR-V(MeFuc)	+[g]	Unknown	Staehelin *et al.* (1994a)
		NodNGR-V (MeFuc,S)	–[g]	Unknown	
		NodNGR-V (MeFuc,Ac)	–[g]	Unknown	
	M. sativa/V. sativa	NodSm-V (C16:2)	+	LCO-III (C16:2)/LCO-IV (C16:2)	Staehelin *et al.* (1994b)
		NodSm-V (C16:2,S)	+	LCO-III (C16:2)	
		NodSm-IV (C16:2)	+	LCO-III (C16:2)	
		NodSm-IV (C16:2,S)	–	–	
		NodSm-III (C16:2)	–	–	
		Chitobiose	–	–	
		Chitotriose	+	–	
CHIT36[h]	*M. sativa*	NodSm-IV (Ac,C16:2,S)	–	–	Minic *et al.* (1998)
		NodSm-IV (C16:2,S)	–	–	
		NodSm-IV (C16:2)	nt	–	
		NodSm-V (C16:2,S)	+	LCO-III (C16:2)	
		Chitin	+	–	
Chi32	*N. tabacum*	NodSm-V(C16:2,S)	+	LCO-III (C16:2)	Schultze *et al.* (1998)
		NodSm-IV (C16:2,S)	–	–	
		NodSm-IV (C16:2)	+	LCO-III(C16:2)	
		NodSm-IV (Ac,C16:2)	–	–	
		LCO-III (C16:2)	–	–	
		Chitin	+	–[i]	Brunner *et al.* (1998)
		Chitohexaose	+	–	
		Chitopentaose	+	–	
		Chitotetraose	+	–	
		Chitotriose	+[j]	–	
		Chitobiose	–	–	
		NodRlv-V (Ac,C18:4)	+	LCO-IV (Ac,C18:4)/LCO-III (Ac,C18:4)	Ovtsyna *et al.* (2000)
		NodRlv-IV (Ac,C18:4)	–		
		LCO-V (Ac,C18:4,Fuc)	+	LCO-III (Ac,C18:4)[k]	
		LCO-IV (Ac,C18:4,Fuc)	–	–	Schultze *et al.* (1998)
Chi30	*M. sativa* [l]	NodSm-V(C16:2,S)	–	–	
		NodSm-IV (C16:2,S)	–	–	
		NodSm-IV (C16:2)	+	LCO-III(C16:2)	
		LCO-III (C16:2)	–	–	
Chitinase class II					
PR-P	*N. tabacum*	NodSm-V (C16:2,S)	+	LCO-III(C16:2)	Schultze *et al.* (1998)
		NodSm-IV (C16:2,S)	-	–	
		NodSm-IV (C16:2)	+	LCO-III(C16:2)	
		LCO-III (C16:2)	–	–	
		Chitin	+	–[m]	Brunner *et al.* (1998)
		Chitohexaose	+	–	
		Chitopentaose	+	–	
		Chitotetraose	+[j]	–	

Table III. *Continued*

Enzyme type[b]	Plant origin	Substrates[c]	Degradation	Acylated reaction products	Reference
		Chitotriose	–	–	
		Chitobiose	–	–	
Chitinase class III					
Srchi13	*S. rostrata*	NodARc[n]	+	Unknown[o]	Goormachtig *et al.* (1998)
Srchi24	*S. rostrata*	NodARc[n]	–	–	Goormachtig *et al.* (2001)
lys28a[q]	*N. tabacum*	NodSm-V (C16:2,S)	+	LCO-III (C16:2)	Schultze *et al.* (1998)
		NodSm-IV (C16:2,S)	+[j]	LCO-II (C16:2)	
		NodSm-IV (C16:2)	+[j]	LCO-III (C16:2)/LCO-II (C16:2)	
		LCO-III (C16:2)	+[j]	LCO-II (C16:2)	
		Chitin	+	–[p]	Brunner *et al.* (1998)
		Chitohexaose	+	–	
		Chitopentaose	+	–	
		Chitotetraose	+[j]	–	
		Chitotriose	+[j]	–	
		Chitobiose	–	–	
		NodRlv-V (Ac,C18:4)	+	LCO-IV (Ac,C18:4)/LCO-III (Ac,C18:4)	Ovtsyna *et al.* (2000)
		NodRlv-IV (Ac,C18:4)	+	LCO-III (Ac,C18:4)/LCO-II (Ac,C18:4)	
		LCO-V (Ac,C18:4,Fuc)	+	LCO-III (Ac,C18:4)	
		LCO-IV (Ac,C18:4,Fuc)	–	–	
lysbl[r]	*N. tabacum*	NodSm-V (C16:2,S)	+	LCO-II (C16:2)/LCO-III (C16:2)	Schultze *et al.* (1998)
		NodSm-IV (C16:2,S)	+	LCO-II (C16:2)	
		NodSm-IV (C16:2)	+	LCO-II (C16:2)/LCO-III(C16:2)	
		LCO-III (C16:2)	+[j]	LCO-II (C16:2)	
		NodSm-IV (Ac,C16:2,S)	–	–	
		NodSm-V (Ac,C16:2,S)	+	LCO-III(Ac,C16:2)	
Chitinase class IV					
CH4[s]	*B. vulgaris*	NodSm-V (C16:2,S)	+[j]	LCO-III (C16:2)	Schultze *et al.* (1998)
		NodSm-IV (C16:2,S)	–	–	
		NodSm-IV (C16:2)	+	LCO-III (C16:2)	
		LCO-III (C16:2)	–	–	
Chitinase class V					
CBP20	*N. tabacum*	NodSm-V (C16:2,S)	+	LCO-III (C16:2)	
		NodSm-IV (C16:2,S)	–	–	
		NodSm-IV (C16:2)	+	LCO-III (C16:2)	
		LCO-III (C16:2)	–	–	
		Chitin	+	–[i]	Brunner *et al.* (1998)
		Chitohexaose	+	–	
		Chitopentaose	+	–	
		Chitotetraose	+	–	
		Chitotriose	–	–	
		Chitobiose	–	–	
Chitinase class VI					
Pz[t]	*N. tabacum*	NodSm-V (C16:2,S)	+	LCO-III (C16:2)	Schultze *et al.* (1998)
		NodSm-IV (C16:2,S)	–	–	
		NodSm-IV (C16:2)	–	–	
		LCO-III (C16:2)	–	–	
		Chitin	–	–	Brunner *et al.* (1998)
		Chitohexaose	+	–	
		Chitopentaose	+	–	
		Chitotetraose	+	–	

Table III. *Continued*

Enzyme type[b]	Plant origin	Substrates[c]	Degradation	Acylated reaction products	Reference
		Chitotriose	–	–	
		Chitobiose	–	–	
Chitinase/lysozyme					
CHIT24[u]	*M. sativa*	NodSm-IV (Ac,C16:2,S)	+	LCO-III (Ac,C16:2)	Minic *et al.* (1998)
		NodSm-IV (C16:2,S)	+	LCO-III (C16:2)	
		NodSm-IV (C16:2)	+	LCO-III (C16:2)	
		NodSm-V (C16:2,S)	+	LCO-III (C16:2)	
		Chitin	+	–	
Nod factor hydrolase					
"Dimer-forming hydrolase"	*M. sativa*	NodSm-IV (C16:2,S)	+	LCO-II (C16:2)	Staehelin *et al.* (1995)
		NodSm-V (C16:2,S)	+	LCO-II (C16:2)	
		NodSm-IV (C16:2)	+	LCO-II (C16:2)	
		LCO-III (C16:2)	–	–	
		NodSm-IV (Ac,C16:2,S)	+/–[v]	LCO-II (Ac,C16:2)	
		NodSm-V (Ac,C16:2,S)	+/–[v]	LCO-II (Ac,C16:2)	
		Material 2[w]	–	–	Staehelin *et al.* (2000)
Whole roots					
	M. sativa/V. sativa	NodSm-V (C16:2)	+	LCO-II (C16:2)[x]/LCO-III (C16:2)/LCO-IV (C16:2)	Staehelin *et al.* (1994b)
		NodSm-V (C16:2,S)	+	LCO-II (C16:2)/LCO-III (C16:2)	Staehelin *et al.* (1995)
		NodSm-IV (C16:2)	+	LCO-II (C16:2)/LCO-III (C16:2)	
		NodSm-IV (C16:2,S)	+	LCO-II (C16:2)	
		LCO-III (C16:2)	+	LCO-II (C16:2)[y]	
		NodSm-IV (C16:0,S)[z]	+	LCO-II (C16:2)	
	V. sativa	NodRlv-V (Ac,C18:4)	+	LCO-IV(Ac,C18:4)/LCO-III(Ac,C18:4)/LCO-II(Ac,C18:4)[a′]	Heidstra *et al.* (1994)
	P. sativum	NodRlv-V (Ac,C18:4)	+	Unknown	Geurts *et al.* (1997)

[a]Nod factors, chitooligosaccharides, or derivatives were incubated with purified enzymes or with whole roots. Resulting acylated reaction products were studied.
[b]Classification according to Collinge *et al.* (1993) and Neuhaus *et al.* (1996).
[c]Nod factors, chitooligosaccharides, or derivatives were used as substrates. Nod factor nomenclature and abbreviations as in Table I.
[d]If no particular name is mentioned, these enzymes were purified but not named.
[e]Mixtures of NodNGR factors as mentioned in Table II.
[f]This particular substrate is degraded (+) or not (–). nt indicates that this substrate was not tried in the degradation assays.
[g]In these cases, Nod factor degradation was quantified by the capacity to induce an extracellular alkalinization in tomato suspension-cultured cells.
[h]CHIT36 does not show any lysozyme activity.
[i]Major degradation products after 24 h of incubation were chitobiose and -triose.
[j]Weak degradation, defined as 20% or less of the substrate, was degraded after 3 h incubation.
[k]LCO-III (Ac,C18:4) is only formed after prolonged incubation.
[l] Chi30 purified from *V. sativa* and *P. vulgaris* exhibit exactly the same characteristics as Chi30 of *M. sativa*.
[m]Major degradation products after 24 h incubation were chitobiose, -triose, and -tetraose.
[n]A mixture of *A. caulinodans* ORS571 Nod factors was used, corresponding to the PI fraction (Mergaert *et al.*, 1997).
[o]Based on thin-layer chromatography pattern analyses, the degradation product may predominantly have a tetrameric and trimeric nature.
[p]Major degradation products after 24 h of incubation were monomers, chitobiose, -triose, and -tetraose.
[q]Protein AC of *Cicer arietinum* degraded the first four substrates in a similar way.
[r]Degradation of chitin and chitooligosaccharides was as for lys28a; SE2 from *Beta vulgaris* and lysb2 from *N. tabacum* showed overall similar activities as lysb1.
[s]CH3 of *B. vulgaris* showed similar characteristics as CH4. Two other class IV chitinases, SP2 and Chi32 of *B. vulgaris* and *Daucus carota*, respectively, differed from CH3 in that the degradation efficiency of NodSm-V (C16:2,S) was much higher than that of NodSm-IV (C16:2).
[t]ChiA of *Serratia marcescens* did not belong to class VI chitinases but exhibited similar characteristics as Pz.
[u]CHIT24 has a strong lysozyme activity.
[v]Relative degradation efficiencies for NodSm-IV (Ac,C16:2,S) and NodSm-V (Ac,C16:2,S) were 23 and 64, respectively, when that of NodSm-IV (C16:2,S) was set at 100%.
[w]Material 2 contained a mixture of GlcN(C16:2)-GlcNAc-GlcNH$_2$-GlcNAc(OH)(S), GlcN(C16:2)-GlcNAc-GlcNH$_2$-GlcNH2(OH)(S), GlcN(C16:2)-GlcNH$_2$-GlcNH$_2$-GlcNH$_2$(OH)(S), GlcN(C16:2)-GlcNAc-GlcNAc-GlcNH$_2$(OH)(S), and GlcN(C16:2)-GlcNH$_2$-GlcNH$_2$-GlcNAc(OH)(S), derived from an enzymatic N-deacetylation of NodSm-IV (C16:2,S).
[x]Kinetics for trimer release were similar for *M. sativa* and *V. sativa* roots, but production of dimeric degradation products was significantly slower when *V. sativa* roots were used.
[y]Formed after prolonged incubations.
[z]Only tested on *M. sativa* roots.
[a′]Based on thin-layer chromatography analyses.

at the reducing-terminal residue of pentameric and tetrameric NodSm factors protect the nearest β-1,4-glycosidic bond against cleavage by a class I chitinase of *M. sativa* (Staehelin *et al.*, 1994b). LCO-III (C16:2) cleavage products are approximately 100,000-fold less active when compared with NodSm-IV (C16:2,S) as determined in a root hair deformation assay on *M. sativa*, suggesting that Nod factor degradation by chitinases generally inactivates Nod factors (Staehelin *et al.*, 1994b).

Remarkably, on *M. sativa* roots, Nod factors induce their own breakdown by enhancing the production of a "dimer-forming hydrolase" (Staehelin *et al.*, 1995). The presence of an *O*-acetyl group at the nonreducing-terminal residue, but not a sulfate ester at the reducing-terminal residue, protects Nod factors against degradation by the dimer-forming hydrolase. This Nod factor hydrolase is strongly induced by NodSm-IV (Ac,C16:2,S), whereas NodSm-IV (C16:2), LCO-III (C16:2), chitotetraose, or chitopentaose have no effect. Possibly, a rapid Nod factor–induced degradation of Nod factors may be part of Nod factor signaling, required for induction of plant genes involved in nodule development, or necessary to finely regulate Nod factor amounts and to avoid a continuous stimulation of the Nod factor perception system(s) (Staehelin *et al.*, 1995), eventually eliciting defense-like reactions (Savouré *et al.*, 1997). Also *P. sativum* roots respond to NodRlv factors and their fucosylated derivatives by an enhanced production of a dimer-forming Nod factor hydrolase (Ovtsyna *et al.*, 2000) (Tables II and III). As illustrated in Table III, a fucosyl group at the reducing-terminal residue of NodRlv factors renders the neighboring β-1,4-glycosidic linkage inaccessible for cleavage by the *N. tabacum* class I chitinase Chi32 and class III chitinase lys28a (Ovtsyna *et al.*, 2000). The importance of the *O*-acetyl group at the nonreducing-terminal residue for protection against degradation has been demonstrated by Chi32, which degrades NodSm-IV (C16:2), but not NodSm-IV (Ac,C16:2) (Schultze *et al.*, 1998) (Table III). Finally, Staehelin *et al.* (2000) showed that a partial de-N-acetylation of NodSm-IV (C16:2,S) rendered the compounds more resistant against degradation by the dimer-forming hydrolase. A mixture of de-N-acetylated compounds (Table III) was approximately 10,000-fold less active in inducing root hair deformation on *M. sativa* roots and does not increase production of the dimer-forming hydrolase. However, Nod factor–induced responses are not inhibited in the presence of de-N-acetylated compounds, indicating that the latter products are probably not perceived (Staehelin *et al.*, 2000).

Nod factor localization

Little is known about the *in situ* localization of Nod factors and their eventual degradation products. Philip-Hollingsworth *et al.* (1997) synthesized *R. leguminosarum* bv. *trifolii* Nod factor analogs that carry the 7-nitrobenzo-2-oxa-1,3-diazole (NBD) group at the C1 position of the reducing-terminal residue. During nodule initiation on *T. repens*, fluorescence has been observed in the cell wall and the plasma membrane of root hairs and other epidermal cells of the young part of the root. Part of the label is seen in the vicinity of the nucleus, another fraction at the root hair base, and later in underlying cortical cells. NBD-related fluorescence has been detected within four to five underlying cortical cell layers, but NBD-labeled chitotriose is not taken up by plant cells (Philip-Hollingsworth *et al.*, 1997).

In an independent study, fluorescent *R. leguminosarum* bv. *trifolii* Nod factor analogs have been synthesized by deacetylating chitotetraose using NodB, thus creating a free amino group subsequently acylated with a commercially available fluorophore-carrying Bodipy-C16:0 fatty acid (Gadella *et al.*, 1997). By using LCO-IV (Bodipy-C16:0), fluorescence has been noticed predominantly at the level of the root hairs of *V. sativa*, which confirms earlier observations using [^{3}H]-NodRlv-V (Ac,C18:0) (Heidstra *et al.*, 1994). The binding is not restricted to root hairs and epidermal cells of the susceptible zone but encompasses also mature and young actively growing root hairs (Heidstra *et al.*, 1994). In addition, an increased labeling has been observed in root hair tips (Gadella *et al.*, 1997). By using a variety of Bodipy-fatty acid–labeled Nod factor analogs and advanced microscopy techniques, it has been shown that Nod factors have a high tendency to be inserted into micelles or vesicles and to be transferred from vesicles to root hair cell walls (Goedhart *et al.*, 1999). Already 3 h after application of labeled Nod factors to *V. sativa* roots at a final concentration of 10^{-8} M, most Nod factors accumulate in the cell wall of root hairs. Occasionally, a very low level of fluorescence has been detected in the plasma membrane. The Nod factor–induced outgrowth, however, is not fluorescent in its cell wall, suggesting that Nod factors are immobilized or that Nod factor migration is slow. Only at high Nod factor concentrations (10^{-6} M) do Nod factors also accumulate in the plasma membrane (Goedhart *et al.*, 2000).

Antibodies raised against NodSm-IV (C16:2,S) have been used for *in situ* visualization of Nod factors during nodule development on *M. sativa* (Timmers *et al.*, 1998). Signals are predominantly present in infection threads, but are also in the cytoplasm of invaded cells and in bacteroids throughout all stages of development. Immunolocalization on nodule sections that exhibit infection threads, internalization events, and infected cells suggests that Nod factors may be internalized in the cytoplasm while bacteria are internalized in plant cells. The spatial-temporal characteristics of this proposed internalization of Nod factors are identical to those of microtubular cytoskeleton disorganizations, which occur when bacteria are taken up by plant cells after release from infection threads. Moreover, the latter microtubular rearrangements have not been observed in bacteria-free nodules. Thus, the concomitant occurrence of internalization of Nod factors and microtubular rearrangements suggests that Nod factors possibly control cytoskeleton changes, which direct the differentiation of bacteria-containing cells (Timmers *et al.*, 1998).

Nod factor perception by putative receptors

Many indications support the hypothesis that Nod factors are perceived by plant receptors. Final concentrations of Nod factors as low as 10^{-9} to 10^{-12} M provoke particular responses on roots, often requiring a defined Nod factor structure (Table II). Valuable information concerning putative Nod factor receptors has been obtained by a study of invasion phenotypes of *S. meliloti nodL* and *nodFE* mutants, and a *nodL/nodF* double mutant on *M. sativa* (Ardourel *et al.*, 1994). Dependent on the mutant—and thus on the type of Nod factors—initiation of

infection thread formation and subsequent penetration can be uncoupled from induction of nodule primordia formation. Based on these observations, Ardourel *et al.* (1994) proposed the presence of a signaling receptor that recognizes Nod factors even when the nonreducing-terminal residue is altered and that would pave the way for infection (controlling infection thread growth), and an entry receptor that demands more stringent structural requirements for recognition, allowing rhizobial ingestion and control of the door opening.

Many attempts have been undertaken to identify putative Nod factor receptors. Some excellent reviews dealing with Nod factor perception have been published recently (Niebel *et al.*, 1999; Cullimore *et al.*, 2001; Oldroyd, 2001).

Nod factor–binding sites

Tritiated NodSm-IV (Ac,C16:2,S) has been used to identify Nod factor–binding sites (NFBSs) in particular fractions of *M. truncatula* roots. Binding is saturable and reversible but independent of the presence of an *O*-acetyl group at the nonreducing-terminal residue, a sulfate ester at the reducing-terminal residue, or an unsaturated fatty acid. Nevertheless, chitotetraose is a poor competitor. This NFBS has been designated NFBS1 (Bono *et al.*, 1995). Two NFBSs for [^{35}S]NodSm-IV (Ac,C16:2,S) have been found in microsomal fractions of *M. varia* suspension-cultured cells, one corresponding most probably to NFBS1, the other designated NFBS2. Initial characterization of NFBS2 showed a higher affinity for NodSm-IV (Ac,C16:2,S) and chitotetraose was a poor competitor (Niebel *et al.*, 1997). An *O*-acetyl group and a C4-hydroxyl group at the nonreducing-terminal residue, but not a sulfate ester at the reducing-terminal residue, are determinants for high-affinity binding to NFBS2. The length of the fatty acid, but not the number or position of double bonds, is important for efficient binding. Also the oligomerization degree of the Nod factor chitooligosaccharide backbone influences the affinity for binding to NFBS2 (Gressent *et al.*, 1999). Recently, a novel protein P60 has been purified from *M. sativa* roots with a high affinity for GlcNAc but also for NodSm factors because the latter are strong competitors for binding of GlcNAc monomers (Minic *et al.*, 2000).

G protein–coupled receptors

The cleavage of phosphatidylinositol (4,5)-biphosphate into inositol (1,4,5)-triphosphate and diacylglycerol (DAG) is a common feature of signal transduction pathways in animals and is often catalyzed by heterotrimeric GTP-binding regulatory protein (G protein)–mediated activation of specific phospholipase C (PLC) isoenzymes. Inositol (1,4,5)-triphosphate can release Ca^{2+} from internal calcium stores, whereas DAG is converted into phosphatidic acid (PA) by DAG kinase. Alternatively, PA is formed by G protein-mediated activation of phospholipase D (PLD), by which structural lipids, including phosphatidyl choline, are hydrolyzed to produce PA. PA kinase converts PA into diacylglycerol pyrophosphate (see den Hartog *et al.*, 2001).

The rapid activation of the Nod factor-induced *MtENOD12* promoter (Table II) has been used to study components of Nod factor signal transduction in alfalfa transgenic lines (Pingret *et al.*, 1998). Nod factor–induced *MtENOD12* expression can be mimicked by mastoparan, an amphipathic tetradecapeptide that activates animal G proteins. Pertussis toxin, an antagonist of G protein activation, and neomycin, a PLC antagonist, block both Nod factor– and mastoparan-induced *MtENOD12* expression. Interestingly, exposure of roots to Nod factors for 15 min is sufficient to activate downstream processes. Furthermore, the release of Ca^{2+} from both internal stores and the external environment is required for *MtENOD12* expression. These indications strongly suggest a role for heterotrimeric G proteins during Nod factor–induced *MtENOD12* expression. Because in animal cells, G proteins are almost invariably coupled to a family of seven transmembrane span receptors, Nod factors may be perceived by such putative plant receptors (Pingret *et al.*, 1998).

Recently, den Hartog *et al.* (2001) further illustrated the importance of G protein–mediated signal transduction pathways in Nod factor– or mastoparan-induced root hair deformation on *V. sativa*. Both Nod factors and mastoparan increase the level of PA, which was caused by increased DAG kinase and PLD activity. In addition, Nod factor– and mastoparan-induced PA production was inhibited by neomycin, a PLC inhibitor, or primary butyl alcohols, which block PLD activity, confirming the involvement of PLC and PLD activities, respectively.

In conclusion, at least one of the Nod factor–induced downstream signaling pathways may involve G protein–mediated activation of both PLC and PLD, leading to the release of Ca^{2+} from internal stores, required for Nod factor–induced *MtENOD12* expression in *M. sativa* (Pingret *et al.*, 1998) and root hair deformation in *V. sativa* (den Hartog *et al.*, 2001).

Lectins and apyrases

Carbohydrate-binding lectin proteins are good candidates to function as putative Nod factor receptors. Transgenic *T. repens* hairy roots expressing a pea lectin gene (*PSL*) are nodulated by the pea symbiont *R. leguminosarum* bv. *viciae* (Díaz *et al.*, 1989); when they carry a *PSL* analog mutated in its sugar-binding site, they can form only pseudonodules after inoculation with *R. leguminosarum* bv. *viciae*, illustrating the importance of the lectin sugar-binding domain (van Eijsden *et al.*, 1995). The presence of the *nodABCIJ*, *nodD*, and *nodFEL* genes in *R. leguminosarum* bv. *viciae* is necessary for nodule development on transgenic white clover roots that express *PSL*, suggesting that *PSL* may be involved in the recognition of *R. leguminosarum* bv. *viciae* Nod factors (Díaz *et al.*, 1995). Recently, *PSL* has been introduced into hairy roots of *T. pratensis* to test several purified Nod factors, including NodRlt-IV/V (Ac,C18:2/C18:4), NodRlv-V (Ac,C18:4), NodRlv-IV (Ac,C18:4), NodRlv-V (Ac,C18:1), NodSm-IV (Ac,C16:2,S), and NodMl-V (Me,Cb,C18:0/C18:1, AcFuc), for their capacity to trigger cortical responses. All of these Nod factors induce cortical cell division with the formation of structures resembling nodule primordia as a result, but a similar response is triggered by chitobiose, chitotriose, chitotetraose, and chitopentaose (Díaz *et al.*, 2000).

On transgenic *Lotus corniculatus* true nodules are formed that express the soybean lectin gene (*SBL*) upon inoculation with *B. japonicum*, a symbiont of soybean, but not of *L. corniculatus*. *SBL* has been localized in *L. corniculatus* root hairs. Mutation of the lectin sugar-binding site abolishes infection thread formation and nodulation by *B. japonicum*. By inoculating a variety of purified Nod factors on both wild-type and transgenic plants, Nod factor–induced nodule primordium formation is found to be influenced not directly by the presence of *SBL*.

Seemingly, a component of extracellular polysaccharides of *B. japonicum*, rather than Nod factors, extends the host range to transgenic *L. corniculatus* plants (van Rhijn *et al.*, 1998). Inoculation of *SBL* and *PSL* transgenic *M. sativa* lines with *B. japonicum* and *R. leguminosarum* bv. *viciae*, respectively, leads to the formation of nodule-like structures only when *S. meliloti* Nod factors are produced (van Rhijn *et al.*, 2001). In the first case, only empty nodule-like structures are observed devoid of infection threads, whereas in the latter infection thread formation appears and some nodule-like structures are infected, but no features indicative for nitrogen fixation are seen. Interestingly, the production of extracellular polysaccharides is a prerequisite for both induction of nodule development and infection thread formation (van Rhijn *et al.*, 2001).

A lectin-nucleotide phosphohydrolase (LNP) has been isolated from roots of *Dolichos biflorus*, a legume that can be nodulated by *B. japonicum* and *Sinorhizobium* sp. NGR234. DbLNP-chitin binding is inhibited by high concentrations of GlcNAc monomers. D-N-acetylated chitooligosaccharides are not, but chitobiose, chitopentaose, and chitohexaose are strong competitors as well as Nod factors purified from *B. japonicum* and *Sinorhizobium* sp. NGR234, illustrating that DbLNP can bind Nod factors. DbLNP is unique because it is not significantly similar with other known lectins and is an apyrase that can hydrolyze a phosphate group from ATP and ADP residues. In the presence of Nod factors, the enzymatic activity of DbLNP increases. Strikingly, DbLNP is localized in the epidermal cell surface of root hairs and DbLNP antiserum blocks root hair deformation and nodule formation, an inhibitory effect that happens only in the antiserum-treated part of the root (Etzler *et al.*, 1999). These observations suggest a role for DbLNP in Nod factor perception by *D. biflorus* roots. Closely related orthologs of DbLNP have been found in *M. sativa* and *P. sativum*, whereas a second apyrase-encoding gene isolated from *D. biflorus*, designated *apyrase-2*, corresponds to sequences in *L. japonicus*, *M. sativa*, and *A. thaliana* (Roberts *et al.*, 1999). The differential distribution of DbLNP along the surface of the root axis coincides with the nodulation zone on *D. biflorus* roots. DbLNP is present on the surface of young and emerging root hairs and is redistributed in response to inoculation with a rhizobial symbiont or to application of Nod factors. The redistribution correlates with the localization of rhizobia on the root hair surface (Kalsi and Etzler, 2000). Two apyrase-encoding cDNAs of *G. soja*, *GS50* and *GS52*, have been characterized. The level of *GS50* expression is not influenced by *B. japonicum*, and GS50 is localized in the Golgi apparatus. In contrast, expression of *GS52*, a DbLNP ortholog, is enhanced rapidly by *B. japonicum*. Anti-GS52 but not anti-GS50 antibodies block nodulation by *B. japonicum*, suggesting a role for GS52 in soybean nodulation (Day *et al.*, 2000).

Chitinase-like proteins

A chitinase homolog, Srchi24, has been isolated from *S. rostrata* (Goormachtig *et al.*, 2001) and its transcript levels increase 4 h after inoculation with Nod factor–producing azorhizobia. Both Srchi24 transcripts and proteins are located in the outer cortical cell layers of developing nodules. An important catalytic glutamic acid residue is replaced in Srchi24, which possibly explains the lack of chitinase activity of the fusion protein between the maltose-binding protein and Srchi24. Currently, research is being performed on the Srchi24 and Nod factor binding (Van de Velde and Holsters, personal communication). Interestingly, a chitinase-related receptor-like kinase (CHRK1) has been isolated from tobacco (Kim *et al.*, 2000) that consists of a C-terminal kinase domain and a putative extracellular domain, which is closely related to class V chitinases of tobacco. The chitinase domain lacks the glutamic acid residue required for chitinase activity, and chitooligosaccharides of chitin are not degraded. CHRK1 is located in plasma membrane fractions, in agreement with a putative role in perception (Kim *et al.*, 2000).

Others

Perhaps the best Nod factor receptor candidate is *sym10*. A *sym10* mutant of pea is Nod$^-$, but infection by mycorrhizal fungi is not affected. Moreover, the *sym10* mutation affects early Nod factor responses, such as [Ca^{2+}] spiking and root hair deformation (Walker *et al.*, 2000).

Finally, *nn1* of alfalfa, associated with a Nod$^-$ phenotype, has been map-based cloned. *nn1* encodes a receptor kinase with a leucine-rich repeat region in the putative external domain and may also serve as a candidate Nod factor receptor (Endre *et al.*, 2001).

Nod factor–like molecules in eukaryotes

Because Nod factors can switch on a complex developmental program leading to nodule formation, it is interesting to investigate whether Nod factor–like compounds are also present in (non)legume plants or other organisms and to decipher eventual Nod factor–related responses.

Transgenic tobacco plants that express *nodA*, *nodB*, or both are severely affected in development, as demonstrated by reduced growth and internode distance, rounded and wrinkled leaves, and compact inflorescence. These observations suggest that tobacco contains substrates that can be modified by NodA or NodB and that are involved in plant growth and organ development (Schmidt *et al.*, 1993).

Arrested embryo development in *Daucus carota* (carrot) cell lines can be rescued by applying 10^{-9} M NodRlv-V (Ac,C18:4) or 10^{-8} M NodRlv-V (Ac,C18:1), whereas addition of chitopentaose was ineffective, suggesting a role of Nod factor–like molecules in carrot development (De Jong *et al.*, 1993). More recently, a mixture of *Sinorhizobium* sp. NGR234 Nod factors has been shown to stimulate *Picea abies* (Norway spruce) protoplast division and regeneration of proembryonic masses from the protoplasts (Dyachok *et al.*, 2000).

In transgenic *Oryza sativa* (rice) plants, harboring an *MtENOD12–gus* fusion, the *MtENOD12* promoter can be activated by either sulfated or nonsulfated Nod factor mixtures purified from *Sinorhizobium* sp. NGR234 but not by chitotetraose. *MtENOD12* promoter activity is observed in cortical parenchyma, endodermis, and pericycle. NodNGR factors, however, do not induce rice root hair deformations, and *MtENOD12* expression is not detected in epidermal cells. Thus, part of the Nod factor signal transduction pathway, required for inducing *MtENOD12* expression, exists in rice plants (Reddy *et al.*, 1998).

Xenopus laevis carries a *DG42* gene that is similar to *nodC* of rhizobia and that is expressed during a short period in embryo development. *In vitro DG42*-dependent synthesis of

chitooligomers with an oligomerization degree of 2–6 has been demonstrated, and longer chitooligosaccharides have been produced as well. Possibly, these molecules are important in vertebrate embryogenesis (Semino and Robbins, 1995). Overexpression of the *DG42* gene in *Saccharomyces cerevisiae* demonstrated that DG42 functions as a hyaluronan synthase that utilizes UDP-GlcA and UDP-GlcNAc to form a hyaluronan polysaccharide of approximately 10^6–10^7 Da with a repeating unit equal to (→4)-β-D-GlcA(1→3)-β-D-GlcNAc(1→) (Pummill *et al.*, 1998). *X. laevis DG42* homologs are found in *Brachydanio rerio* (zebrafish), human, and mouse; the *in vitro* synthesis of chitooligosaccharides by zebrafish or mouse extracts from appropriate developmental stages depends on *DG42* (Semino *et al.*, 1996). That chitooligosaccharides are synthesized during particular developmental stages of zebrafish and *Cyprinus carpio* (carp) was confirmed by Bakkers *et al.* (1997), who purified extracts and labeled chitooligosaccharides by an *in vitro* transfucosylation reaction, using GDP-[U-^{14}C]fucose and *B. japonicum* NodZ. Carp produces predominantly chitotetraose and zebrafish chitotetraose and chitopentaose. Strikingly, when fertilized zebrafish eggs are injected with anti-DG42 serum or NodZ protein, but not with rabbit preimmune serum, severe malformations in trunk and tail have been observed (Bakkers *et al.*, 1997). A similar phenotype is apparent when the *B. japonicum nodZ* gene is expressed in one-cell zebrafish embryos (Semino *et al.*, 1998), and when a *Streptomyces plicatus* chitinase 63 is injected (Semino and Allende, 2000). These observations suggest that chitooligosaccharides may play a role in the development of these vertebrates.

Nod factor modeling

Information about the 3D structure of Nod factors is needed to understand the role of substitutions in biological activity. LCO-IV (C18:1,MeFuc) and LCO-IV (C16:1,S) have been analyzed by nuclear magnetic resonance spectroscopy (Gonzalez *et al.*, 1999), and a study of the crystal structure of NodNGR factors is currently under investigation (Broughton, personal communication). Because these techniques are laborious and their application on many samples is practically impossible, 3D modeling may be a useful alternative. Molecular modeling is a fast and reliable method to obtain preliminary structural information. It has recently been applied to cell wall polysaccharides, such as pectins (Pérez *et al.*, 2000). However, the data should be interpreted with care; predictions obtained by modeling may not correspond completely with the real structures, particularly for compounds present in defined microenvironments, such as plant cell walls or cytoplasmic membranes.

A case study has been performed on Nod factors produced by *A. caulinodans* ORS571. As mentioned in Table I, *A. caulinodans* produces mainly pentameric Nod factors with either a C16:0, C18:1, or C18:0 fatty acid at the nonreducing-terminal residue. All Nod factors contain an *N*-methyl and a 6-*O*-carbamoyl group at the nonreducing-terminal residue, and no glycosylations, an L-fucosyl group, a D-arabinosyl group, or both at the reducing-terminal residue (Table I). D'Haeze *et al.* (2000) suggested that carbamoyl and glycosyl groups of azorhizobial Nod factors may play a role in the recognition of Nod factors by putative receptors. A 3D model of a fully substituted NodARc-V (Me,Cb,C18:0,Fuc,Ara) (Figure 2) illustrates that the fatty acid chain is positioned approximately perpendicularly to the chitooligosaccharide backbone. Neither this feature nor the global structure changes significantly when the C18:0 fatty acid is replaced by a C18:1 or C16:0 fatty acid; when the carbamoyl, fucosyl, and/or arabinosyl groups are removed; or when the carbamoyl or fucosyl groups are replaced by an acetyl group or a sulfate ester, respectively. De-N-acetylation of the second, third, fourth, or fifth GlcNAc residue of NodARc-V(Me,Cb,C18:0,Fuc,Ara) does not influence the global structure (data not shown). However, when the *N*-methyl group of NodARc-V(Me,Cb,C18:0,Fuc,Ara) is removed, the Nod factor conformation is severely altered in that the fatty acyl chain is oriented almost in parallel with the chitooligosaccharide backbone (Figure 2). The modifications at the reducing- or nonreducing-terminal residue have no effect, also when a carbamoyl group is substituted at the C3 and/or C4 position (data not shown).

Although no conclusive data about the role of the *N*-methyl group for biological activity of azorhizobial Nod factors are available yet (D'Haeze *et al.*, 2000), the observation, based on Nod factor modeling, suggests that the presence of an *N*-methyl group may be necessary for correct positioning of the fatty acyl chain. Such positioning may be important for Nod factors to be recognized efficiently by their receptors or to be correctly integrated into the cell wall and/or plasma membrane. Recently, Demont-Caulet *et al.* (1999), who synthesized Nod factors carrying a variety of fatty acids, indicated how important a correct hydrophobic tail is for lateral Nod factor

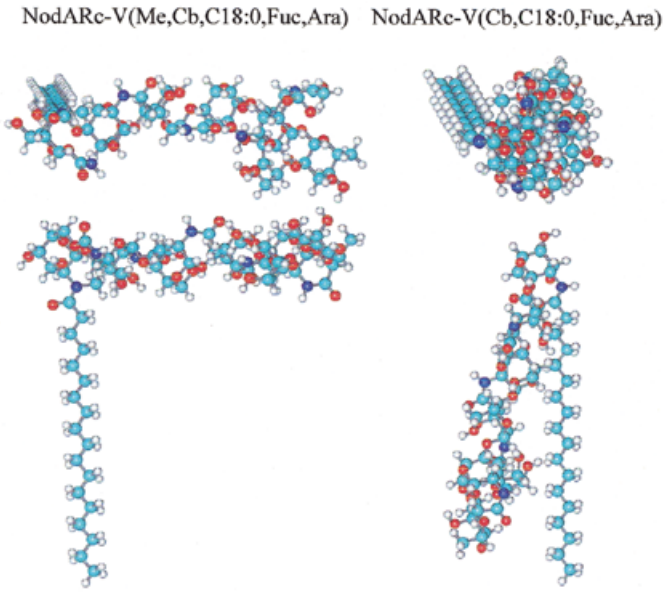

Fig. 2. Nod factor modeling. Modeling of Nod factors and derivatives was performed using the software package HyperChem Release 5.1 (Hypercube, Florida Science and Technology Park). A description of the compounds is indicated above the models (for nomenclature, see Table I). Each model consists of two panels, the upper panel represents a 90° projection of the lower panel around a horizontal axis. All upper and lower models are oriented in approximately a similar way, based on the orientation of the fatty acyl chain. Atoms are indicated: white, hydrogen; pale blue, carbon; dark blue, nitrogen; red, oxygen.

diffusion. In conclusion, Nod factor modeling may provide useful indications to understand how Nod factors may be perceived by the plant.

Some conclusions, leading to many questions

Since the identification of Nod factors, major progress has been made in our knowledge on the molecular mechanisms of controlled bacterial invasion and induction of organ development. Data on nodulation-related gene expression have accumulated steadily over the past decade and are now expected to grow exponentially with the advent of genomics/genetics programs. The next challenges will be data mining and fitting all the data into a functional, biological context. Knowledge from other developmental processes and from studies of pathogen infections provides a solid background for setting up models and hypotheses in the nodulation field.

Some players of the game are known, but the rules of symbiotic development need to be deciphered. An important question, the answer of which will definitely lead to another landmark in symbioses history, is how Nod factors are perceived. Various protein candidates may serve as putative plant Nod factor receptors, and it seems plausible that different Nod factor receptors exist in a particular legume plant, all of them contributing to the final picture. If different Nod factor receptors exist, how is their expression regulated? Where are they expressed? Do they have a specificity for particular Nod factors? Do they govern specific downstream responses? Are the receptors monomers or dimers, or are they part of a more complex perception construction? Based on Nod factor localization and binding studies, the picture emerges that the primary Nod factor perceiving proteins or protein complexes are localized at the cell wall, whereas true Nod factor receptors for downstream signaling may be expected at the plasma membrane. If so, how do Nod factors traverse the cell wall to reach their receptor(s)? Is this a simple diffusion process or do apoplastic adaptor proteins play a role, comparable to the chaperone concept, in transport, protection, or docking with a receptor in the membrane?

The picture of initial Nod factor recognition gets even more complicated, because Nod factors may be degraded by plant-derived Nod factor-hydrolyzing enzymes. What is the turnover of Nod factors in the host environment, and what is the fate of the degradation products? Are these molecules also recognized by the host, and what may be their role during nodule initiation?

What is the sequence of events directly downstream of Nod factor perception? An impressing set of Nod factor–induced early responses have been recorded in different systems, involving Ca^{2+}, membrane potential, and pH. However, a clear picture of causes and consequences is missing, and the order of events and which responses are controlled by which changes are unknown.

Finally, how do Nod factors fit into the more general scheme of oligosaccharide signaling and/or lipid-derived signals in eukaryotes? Rhizobia may mimic fungal chitin signals or endogenous oligosaccharins derived from cell walls or from glycoproteins. For researchers in the symbiosis field, the challenges are numerous and the most tempting ones have yet to come.

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Abbreviations

DAG, diacylglycerol; LCO, lipo-chitooligosaccharides; LNP, lectin-nucleotide phosphohydrolase; NBD, 7-nitrobenzo-2-oxa-1,3-diazole; NFBS, Nod factor–binding site; Nod, nodulation; PA, phosphatidic acid; PLC, phospholipase C; PLD, phospholipase D.

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