Nod Factors and Nodulation in Plants

Irma Vijn, Lucinda das Neves, Ab van Kammen, Henk Franssen, Ton Bisseling

Certain plants—the legumes—are able to reduce nitrogen from the air and form ammonia. This "fixed" nitrogen serves as the most important source of nitrogen for the legume and, secondarily, for other plants and animals. This unique capacity is conferred by the symbiotic bacterium *Rhizobium*, which resides within nodules on plant roots. These bacteria have recently been shown to secrete a class of lipo-oligosaccharides that trigger the early steps of legume nodule formation. These lipo-oligosaccharides may, in fact, be related to as yet unidentified, endogenous plant growth regulators.

The first visible step of the *Rhizobium*-legume interaction is the deformation and curling of root hairs. Within these curled root hairs, rhizobia promote the formation of tubular structures—the so-called infection threads—by which the bacteria enter the plant. As the infection threads develop, mitotic activity is induced in the terminally differentiated root cortex. In temperate legumes like pea, vetch, and alfalfa, these mitotically activated cells, which form the nodule primordia, are located in the inner cortex. The infection threads grow from the root hairs toward these nodule primordia. There they enter primordium cells, where the bacteria move into the cytoplasm by an endocytotic process. Subsequently, the nodule primordium differentiates into a mature nodule, which provides the proper environment for the bacteria to reduce atmospheric nitrogen into ammonia, and the process of symbiotic nitrogen fixation can start.

Each of these steps in nodule formation is marked by the expression of nodule-specific plant genes that code for plant proteins called nodulins (*1*). Even a basic process like cortical cell division is accompanied by the expression of the nodulin genes *ENOD12* (*1*) and *ENOD40* (*2*). Although the function of these genes in these dividing plant cells is unknown, these nodulins allow easy identification of *Rhizobium*-induced cell divisions when they are compared to other dividing plant cells.

The *Rhizobium* genes of prime importance in the early steps of nodulation are the *nod* genes (*3–5*). In free-living bacteria these genes are not expressed, with the exception of the *nodD* gene. Flavonoids secreted by the plant in concert with the NodD protein induce the expression of the other *nod* genes. Subsequently, the encoded Nod proteins cause the production of signal molecules: mono-*N*-acylated-chitin oligomers, called Nod factors (Fig. 1). The *nodABC* genes, common to all rhizobia, are required for the production of the core oligosaccharide molecule. The host-specific *nod* genes control the decoration of the core molecule with side groups (Fig. 1), which likely render Nod factors specific for their particular host. Indeed, the Nod factor of *R. meliloti* carries a sulfate group on the reducing sugar, whereas this substitution is lacking on the factors produced by *R. leguminosarum* bv. *viciae*. Normally the Nod factor of *R. meliloti* is not active on vetch, a host plant of *R. leguminosarum* bv. *viciae*, but after removal of the sulfate group it gains the ability to interact with vetch and loses this ability on its own host, alfalfa (*5*). The involvement of Nod factor substitutions in host specificity is emphasized by studies on *Rhizobium* sp. NGR234, which has a broad host range. This bacterium has the unique ability to interact with more than 70 genera of legumes as well as with *Parasponia andersonii*, the only non-legume that can form nodules with *Rhizobium*. *Rhizobium* sp. NGR234 produces over 18 different Nod factors, each having a similar basic structure but with different substitutions (*6*).

Purified Nod factors, when applied to legume seedlings at concentrations as low as 10^{-12} M, stimulate the differentiation of epidermal cells into root hairs and also deform root hairs (*3–5*) (Fig. 2). Nod factors also induce cytoplasmic rearrangements in the root outer cortex, which lead to the formation of cytoplasmic bridges that form a track normally followed by the *Rhizobium*-induced infection thread. The formation of infection threads is not induced by purified Nod factors, but the expression of the early nodulin genes *ENOD5* and *ENOD12*, which are related to the infection process (*1*), is induced in root epidermal cells by Nod factors (*7*). Furthermore, these compounds are able to induce cell divisions and the expression of the early nodulin gene *ENOD40* in the inner cortex of the root, preferentially opposite the proto-xylem poles just as *Rhizobium* does (Fig. 3). In addition, *ENOD40* gene expression is induced in the root pericycle facing the nodule primordia. The *ENOD12* gene is also induced but only in the cells of the primordium (Fig. 3). This spatial pattern of *ENOD12* and *ENOD40* expression induced by purified Nod factors precisely corresponds to the pattern after *Rhizobium* infection. So Nod factors induce cortical cell divisions as well as nodulin gene expression in a spatially controlled manner.

In these induction experiments, the roots are bathed in a medium containing Nod factors, so this defined pattern of gene expression implies that the susceptibility of a root cortical cell to become mitotically active is determined by its position in the root.

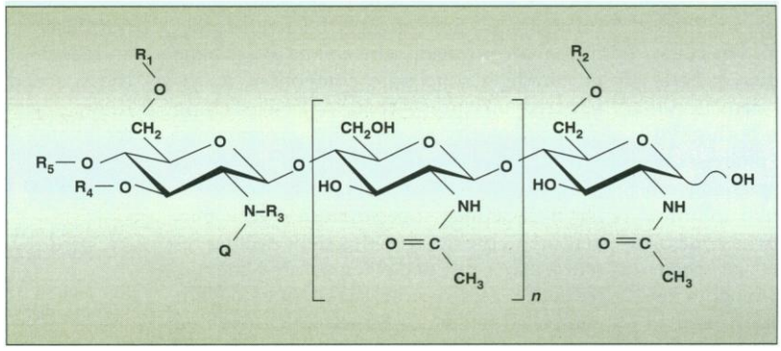

Fig. 1. Structure of Nod factors. The chitin oligomer and the acyl moiety (Q) are present in all Nod factors. The number (*n*) of *N*-acetyl glucosamine residues can vary. Q can vary in length and in the number of unsaturated bonds. Several different substitutions to the sugar backbone occur (R_1 to R_5) (*4–6*).

	R. leguminosarum bv. *viciae*	*R. meliloti*	*R.* sp. NGR234
n	2 or 3	1, 2, or 3	3
Q	C18:1 or C18:4	C16:2	C18:1 or C16:0
R_1	CH_3CO or H	CH_3CO or H	H
R_2	H	SO_3H	2-*O*-methylfucose or substituted with either 3-*O*-CH_3CO or 4-*O*-SO_3H
R_3	H	H	CH_3
R_4	H	H	NH_2CO or H
R_5	H	H	NH_2CO or H

The authors are in the Department of Molecular Biology, Agricultural University, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands.

About two decades ago it was postulated by Torrey and Libbenga (8) that this positional information is controlled by a plant compound (stele factor) released from the proto-xylem poles. A stele factor capable of inducing cell divisions in the inner cortex of pea root explants has since been purified, but its structure remains to be elucidated (9). In view of these observations, it seems likely that the position where cell divisions can be induced in the root cortex is determined by the interplay of at least two oppositely oriented morphogen gradients. One morphogen (stele factor) is released from parts of the plant vascular tissue and the other is the Nod factor itself or a hypothetical secondary signal generated in the plant by the Nod factor.

How do the Nod factors induce their effects? Separate parts of the Nod factors contribute to the biological activity of these compounds (3–5). As discussed above, the presence of a sulfate group on the terminal reducing sugar is a major determinant for the activity on alfalfa. The nature of the lipid moiety is equally important for the activity of the Nod factors. For example, *R. leguminosarum* bv. *viciae* Nod factors containing a C18:4 fatty acid chain are mitotically active, whereas compounds containing a C18:1 group (Fig. 1) are unable to induce cell divisions (4). Since the molecules that induce the initial steps of nodulation have a very specific structure and are active at nanomolar to picomolar concentrations, they probably act through receptors.

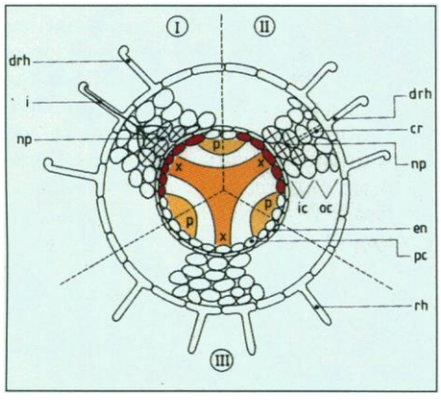

Fig. 2. How nodulation begins. Changes in the root of a temperate legume induced by *Rhizobium* bacteria (part I), purified Nod factors (part II), as compared to the untreated situation (part III). The induction of nodulin gene expression in the pericylce is represented by the red area. P, phloem; np, nodule primordium; i, infection thread; cr, cytoplasmic rearrangements; pc, pericycle; en, endodermis; ic, inner cortex; oc, outer cortex; drh, deformed root hair; rh, root hair; and x, xylem pole.

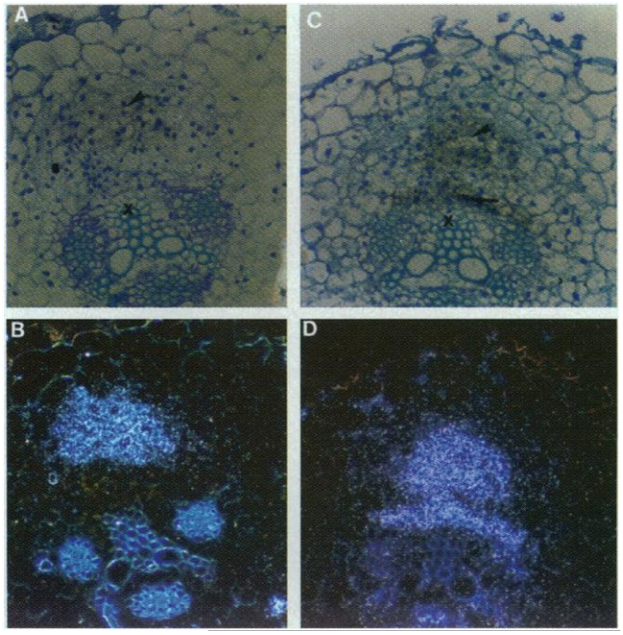

Fig. 3. Gene induction by Nod factors. Cross sections of 6-day-old *Vicia sativa* (vetch) roots treated with purified Nod factors of *R. leguminosarum* bv. *viciae*. *ENOD12* is expressed in the nodule primordia (arrowheads) [bright field (**A**) and dark field (**B**)]. The *ENOD40* gene is expressed in the nodule primordia and the pericycle of the root vascular bundle (arrow) opposite a protoxylem pole (x) [bright field (**C**) and dark field (**D**)]. Labeling was by in situ hybridization with ^{35}S-labeled antisense RNA probes.

The nature of this possible receptor–Nod factor interaction is unclear. Does the plant recognize just one factor or has each factor its own receptor? Is the Nod factor itself or a molecule derived from the Nod factor being recognized? Is only part of the Nod factor active in signal transduction, while the remaining part of the molecule has a function in transporting the Nod factor to the receptive cells? And, with respect to signal transduction, are Nod factors or the active parts of the Nod factors transported by a specific mechanism toward the inner cortical cells or are secondary signal molecules elicited in the epidermis of the root? Further studies to answer these questions will require both biochemical and genetic approaches. The biochemists will focus in particular on the identification of the putative receptor molecule. This work will be facilitated by the chemical synthesis of Nod factors (10), which will allow the labeling of these signal molecules.

The major task for the geneticists will be the identification of useful mutants among the many that have been generated during the last few decades. Several legume mutants have been described that have lost the ability to interact with *Rhizobium*. In a few cases there appears to be a gene-for-gene relation between a plant gene controlling nodulation and a host-specific *nod* gene. The best studied example is the *sym2* gene present in the primitive pea line Afghanistan (11). Afghanistan peas form in general root nodules with *R. leguminosarum* bv. *viciae* strains containing an additional *nod* gene, *nodX* (12). Commercial pea lines carrying *sym2* introgressed from Afghanistan peas form root nodules only with *R. leguminosarum* bv. *viciae* strains containing the extra *nodX* gene and not with common *R. leguminosarum* bv. *viciae* lines that lack a *nodX* gene. Recently, it has been shown that NodX is involved in adding an O-acetyl group at the reducing sugar (R_2) (Fig. 1) (13). Therefore, it can be hypothesized that *sym2* encodes a receptor that specifically recognizes *nodX*-modified Nod factors.

Recently, the laboratory of De Vries (14) has obtained evidence that Nod factors can also influence development in a non-legume plant. They showed that a mutant cell line from carrot, which is arrested in somatic embryogenesis, can be rescued by addition of Nod factors. Thus, plant-produced Nod factor–like molecules can be involved in non-symbiotic, developmental processes in plants, suggesting that these processes may be the evolutionary origin of the bacterial signal molecules. Furthermore, Nod factor recognition mechanisms might occur in non-legume plants, which could allow the use of a model system like *Arabidopsis* to study recognition of Nod factors. In this way, research originally initiated to elucidate the development of nitrogen-fixing root nodules might provide tools to study plant development from an unexpected perspective.

References and Notes

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