Regulation of nodulation in *Rhizobium leguminosarum*

B.J.J. Lugtenberg

Under conditions of nitrogen limitation, *Rhizobium* bacteria infect leguminous plants, which then form root nodules. In this symbiotic process the bacteria present in the nodules (as bacteroids, differentiated forms of the bacteria) fix atmospheric N\(_2\) and convert this into ammonia which is used as a nitrogen source by the plant. In return, the plant provides the bacterium with a carbon source.

Nodulation by *Rhizobium* is a host-specific process: of the various species and biovars of *Rhizobium* only one, or a limited number, is able to nodulate a certain plant and fix nitrogen efficiently. N\(_2\)-fixation is an extremely important process since, after water, nitrogen is the second most important compound limiting for world crop production.

Since 1896, *Rhizobium* strains selected for their N\(_2\)-fixing ability have been commercially used as inoculants, to increase the production of especially soybean and alfalfa. Inoculation is often only moderately successful since indigenous rhizobia are better adapted to the local conditions. They therefore often outcompete the inoculant strains.

The Nodulation Process

The following stages have been established or proposed.

1. Chemotaxis to root exudate.
2. Attachment of bacteria to the root hair tip of the plant.
3. Induction of *nod* genes by flavonoids secreted by the plant. The result is secretion of soluble, low molecular weight components which are involved in hair curling, release of additional *nod*-gene inducers, meristem formation and elicitation of at least one nodulin, ENOD12.
4. Root hair curling leading to internalization of the rhizobia.
5. Infection thread initiation.
7. Growth and branching of infection thread followed by invasion of (dividing) meristemic cells. Bacteria in the infection thread have divided many times.
9. Differentiation of bacteria into bacteroids.
10. Continued meristemic growth resulting in a nodule.
11. Synthesis of nodulins by the plant (a.o. leghemoglobin).
12. Conversion of N\(_2\) into ammonia.

Role of Sym Plasmid

*Rhizobium* strains usually contain one or more plasmids, among which is the Sym(biosis) plasmid. Loss of this plasmid, e.g. by growth at elevated temperatures, results in strains which are no longer able to nodulate. Transfer of another Sym plasmid into such a cured strain restores nodulation ability. Even more interestingly, the host-specificity of the resulting strain is the same as that of the donor strain of the Sym plasmid. Also, if the Sym plasmid is transferred to a cured *Agrobacterium tumefaciens* strain, the resulting strain is able to form nodules, which usually are ineffective.

Based on these data it must be concluded that (i) the Sym plasmid carries important nodulation genes, and (ii) the Sym plasmid determines the host range.

Sym-Plasmid-Localized Nodulation Genes

About 13 nodulation genes are localized on pRL111, the Sym plasmid of the *Rhizobium leguminosarum* bv. *viciae* strain that is mostly used in our studies. Only *nodD*, the positive regulatory gene, is constitutively transcribed, whereas the
operons nodABCJ, nodFEL, nodMNT and nodO are transcribed only upon activation of NodD protein by certain flavonoids.

Many of these nod genes are involved in the production and/or secretion of soluble low molecular weight extracellular compounds as described for R. meliloti by Dénaire's group in Toulouse (Lerouge et al. 1990). With respect to the function of nod genes one has to take into account that some nod genes can functionally replace others, e.g. nodF and nodO (Economou et al. 1990), although NodE protein is localized in the cytoplasmic membrane (Spank et al. 1989), whereas NodO protein is secreted (De Maagd et al. 1989c).

**Research in the Near Future**

**Attachment**

Attachment is independent of the Sym plasmid. So far, three factors have been implicated in attachment of R. leguminosarum bv. viciae bacteria to the tips of pea root hairs: a bacterial protein, bacterial cellulose fibrils and the plant's lectin (Lugtenberg et al. 1989). Once mutants lacking the bacterial protein, rhicadhesin, have been generated, it can be tested whether attachment is a prerequisite for nodulation. Once antibodies are available it can be tested whether rhicadhesin is just a cell surface protein or whether it is organized in fimbriae-like structures. Finally, the receptors of rhicadhesin and of lectin still have to be identified.

**Activation of NodO Protein**

Evidence has been presented for a direct interaction between NodD protein and inducing flavonoids (Spank et al. 1987), presumably in the cytoplasmic membrane (Recourt et al. 1989; Schlaman et al. 1989). This interaction can play a role in host-specificity. Components which inhibit activation of NodD protein have also been identified (Firmin et al. 1986; Djordjevic et al. 1987). It is not clear yet whether they play a role in the nodulation process. It is hard to make a model which includes all reported interactions of NodD protein, namely those with the cytoplasmic membrane (Schlaman et al. 1989), with a flavonoid inducer (Recourt et al. 1989) and with nod box DNA (Fisher et al. 1988).

**The Bacterial Answer upon nod Gene Expression**

Many Sym plasmid-localized nod genes function in the synthesis and/or secretion of soluble low molecular weight molecules. In the case of R. meliloti, one such molecule has been identified as a sulphated β-1,4-tetrasaccharide of D-glucosamine, in which three amino groups are acetylated and one was acylated with a C-16 bis-unsaturated fatty acid (Lerouge et al. 1990). Future research will unfold the identities of similar molecules secreted by other rhizobia. Moreover, these studies will focus on (i) the function of individual nod genes in the synthesis and secretion of these molecules, (ii) the roles of these molecules in hair curling, meristem formation and secretion of additional flavonoid inducers. Of particular interest will be the molecular mechanism of signal perception and transduction, in which ENOD12 elicitation (Scheres et al. 1990) could be used as the response of the signal perception/transduction pathway. Finally, it is tempting to speculate that the bacterial answer molecules may interact with lectin (Diaz et al. 1989) in order to initiate infection thread formation.

**Host-Specificity**

R. leguminosarum bv. viciae and R. leguminosarum bv. trifolii are closely related rhizobia which differ in host-specificity in that the former nodulates pea and Vicia, whereas the latter nodulates clovers. Several molecules are involved in the determination of this host-specificity.

(i) NodD proteins of the two biovars become activated by different sets of flavonoids which largely overlap but which also contain activators of only one of the two NodD proteins (Spank et al. 1987).

(ii) Of the inducible nod genes nodE has been shown to be the only gene that determines the difference in host specificity (Spank et al. 1989).

(iii) When the gene encoding pea lectin is expressed in white clover hairy roots, R. leguminosarum bv. viciae can form effective nodules on the transgenic hairy roots. The same bacterium is unable to form stable infection threads on white clover plants or hairy roots carrying only its own lectin gene (Diaz et al. 1987).

Future research will focus on unravelling the molecular mechanism of host-specificity.

**Functional Common Roles of Different nod Genes**

Mutations in some nod genes have hardly any effect on nodulation except when, in addition, other nod genes are also missing (Van Brussel et al. 1988). The molecular basis of this compensation effect is not understood. The best-known example is the couple nodO/nodE (Economou et al. 1990). NodO encodes a secreted flavonoid-inducible protein (De Maagd et al. 1989c,d) which binds to roots of Vicia hirsuta and V. sativa (Lugtenberg et al. 1990). It binds Ca\(^{2+}\) ions (Economou et al. 1990). NodE encodes a cytoplasmic membrane protein (Spank et al. 1989) which is involved in synthesis or secretion of a low molecular weight soluble molecule which is a major determinant of host-specificity (Spank et al. 1989) and which elicits nodulin ENOD12 synthesis (Scheres et al. 1990). We have interpreted these data as indicating that these are at least two different nodulation pathways (Lugtenberg et al. 1990).
Role of Lipopolysaccharide in Nodulation

The exact stage of the nodulation process in which lipopolysaccharide (LPS) mutants are defective seems to differ between biovars. Biovar phaseoli mutants form abortive infection threads and are not released into bean plant cells (De Maagd & Lugtenberg 1989). Biovar viciae mutants lacking O-antigen are released from the infection thread (although possibly less frequently than wild type cells), and form (some) bacteroid-filled V. hirsuta plant cells (De Maagd et al. 1988, 1989b; Prief 1989). In all these cases the nodules are small, white and devoid of nitrogenase activity. Thus, it appears that complete LPS is not essential for the early steps of symbiosis, but that, depending on the bacterium-host combination, complete LPS is required for normal infection thread development, bacterial release and bacteroid differentiation (De Maagd & Lugtenberg 1989).

One can speculate on the role of LPS at the molecular level (De Maagd & Lugtenberg 1989).

1. LPS may contain, either constitutively or induced, a structure that acts as a signal towards the plant. This signal may be released by a plant enzyme. The signal may (i) be involved in infection thread initiation, (ii) induce endocytosis of the bacteria, or (iii) inhibit plant defence reactions. The enormous variation in O-antigen chain sugar composition make the involvement of this LPS-moiety in the generation of a specific signal not very likely.

2. LPS may contain a structure that acts as a receptor for a plant signal. The interaction may trigger the next step in symbiosis. A possible candidate for a plant cell wall receptor is root lectin (Wolpert & Albersheim 1976). The disadvantage mentioned under (1) for such a specific role also applies here.

3. Normal symbiosis may require a minimal bacterial cell surface density of (lipo)polysaccharide molecules, e.g. because of requirements for surface change or hydrophobicity (De Maagd et al. 1989b). Such a relaxed requirement can be fulfilled by many structurally different (lipopolysaccharides).

4. The effect of LPS mutations on symbiosis may be indirect, e.g. due to the lack of another component which shares part of the same pathway, or due to a role of LPS in outer membrane stability.

Differentiation from Bacterium to Bacteroid

During development of bacteria to bacteroids a number of cell surface changes take place, particularly in LPS and outer membrane protein composition (De Maagd et al. 1989a). The latter two changes are probably initiated in the stage of release from the infection thread (Lugtenberg et al. 1990). Another recent finding is that nod gene expression are switched off by the same (plant) signal and what the nature of this signal is.

References


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