time to identify the carbohydrate-bearing structures. Recent examples of glycoproteins originally
detected with lectins that have now been identified are desmosomal glycoproteins [15] and integrins
[16]. Changes in the carbohydrate moieties of adhe-
sion molecules can alter their function [17–19] and
thus the lectin patterns may turn out to provide new
insights into the changes in the adhesive properties
of keratinocytes that occur during terminal differen-
tiation [20].

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The lipo-oligosaccharidic symbiotic signals of Rhizobium meliloti
Philippe Roche,* Frédéric Debellé,† Patrice Lerouge,* Jacques Vasse,† Georges Truchet,†
Jean-Claude Promé* and Jean Dénarié†

*Centre de Recherches de Biochimie et de Génétique Cellulaire, CNRS-UPS, 118 route of Narbonne, 31602
Toulouse Cedex, France and †Laboratoire de Biologie Moléculaire des Relations Plantes-Microorganismes,
CNRS-INRA, B.P. 27, 31326 Castanet-Tolosan Cedex, France

Introduction
Rhizobia are soil bacteria that elicit the development of root nodules, the plant organs in which the bac-
teria fix nitrogen. These symbiotic associations are specific and a given rhizobial strain nodulates a
limited number of leguminous hosts [1, 2]. For example, Rhizobium meliloti nodulates alfalfa whereas
Rhizobium leguminosarum nodulates pea and vetch. Nodule induction is a complex process determined by sets of bacterial and plant genes and generally involves the following steps: Recognition
between bacteria and the host plant, root hair curling, infection thread formation within root hairs and
the root cortex, induction of cortical cell divisions and nodule organogenesis [2, 3].

The R. meliloti nodulation (nod) genes
The bacterial genes involved in this process are the nodulation (nod) genes [2]. The expression of nod
genes is under the control of regulatory NodD pro-
teins and requires the presence of phenolic com-
 pounds exuded by the host plants [2].

The nodABC genes are referred to as common nod
genes. They are structurally and functionally conserved in all Rhizobium, Bradyrhizobium and
Azorhizobium species studied so far [1, 4]. The
nodABC genes play a crucial role in infection and
nodulation, since a mutation in these genes results
in a complete loss of the ability to elicit any detect-
able plant responses whatever the host, the type of
infection, or the type or location of nodules nor-
mally produced [1–3]. The species-specific nod
genes, such as nodFEG, nodH and nodPQ in R.
meliloti, are involved in defining the rhizobial host
range [1]. A map of this region is shown in Fig. 1. Bacterial strains carrying mutations in these genes display altered infection and nodulation functions including changes in the host range. For example, nodH mutants have lost the ability to infect and nodulate alfalfa but gained the ability to infect and nodulate vetch, which is not normally a host [5-7]. NodQ mutants have an extended host range and nodulate both alfalfa and vetch [8].

**Nod genes determine the production of extracellular Nod factors**

The sterile supernatants of *R. leguminosarum* cultures, grown in conditions of nod gene expression, elicit root hair deformations (Iad) and root thickening and shortening (Tsr) on vetch [9, 10]. *R. meliloti* sterile filtrates elicit hair deformation on alfalfa [11]. Inactivation of the common nodABC genes suppresses these biological activities. Thus the nod genes are involved in the production of extracellular Nod factors. These Nod factors are specific since the filtrates of *R. leguminosarum* are active on vetch and not alfalfa, whereas those from *R. meliloti* are active on alfalfa and not vetch [11].

**R. meliloti** Nod factors are sulphated lipo-oligosaccharides

In *R. meliloti* the extracellular factors are produced in very low amounts. To obtain the quantities required for structural analysis we constructed overproducing strains by manipulating the regulatory circuits of nod gene expression. Increasing the copy number of the nod gene region or of the transcriptional activators syrM and nodD3 resulted in an increase in the production of Nod factor by approximately 1000-fold [12, 13]. Using the alfalfa hair deformation bioassay, Nod factors were purified by a series of preparative and analytical reverse-phase h.p.l.c., ion-exchange and gel permeation chromatography [12, 14]. The structure of the compounds was determined by a combination of n.m.r. spectroscopy, mass spectrometry (m.s.) and various chemical modifications.

The major Nod factors from *R. meliloti* are β,1-4-linked tetra- or pentamers of N-glucosamine, N-acetylated on the terminal non-reducing residue and N-acetylated on the other residues (see Fig. 2). In other words Nod factors are N-acetylated chitin oligomers [12-14]. The molecules are O-sulphated on the C-6 of the reducing aminosugar and may be O-acetylated on the C-6 of the terminal non-reducing end [12-14]. The major N-acyl group is a C16 chain with two double bonds in positions 2 and 9 [12]. Nod factors of similar general structure have recently been found in *R. leguminosarum*: They are also N-acetylated chitin oligomers but differ by the substituents linked to the chitin oligomer backbone. They are not sulphated and the N-acyl group is different [15].

**Biological activity of Nod factors**

Purified Nod factors from *R. meliloti* elicit root hair deformations on lucerne at the very low concentration of 10^−10−10^−12 M [12, 13]. They also elicit cortical cell divisions and the formation of genuine root nodules on lucerne at concentrations down to 10^−13 M [16]. Thus lipo-oligosaccharides of rhizobial origin elicit plan responses at different steps of the symbiotic process, including genuine plant organogenesis. Nod factors have also been shown to induce the transcription of plant genes involved in early steps of nodulation, the early nodulin genes ENOD5 and ENOD12 [3, 17], at concentrations as low as 10^−13 M. These factors are thus active at concentrations clearly lower than those at which the previously characterized growth regulators are active. Chemical and genetic modifications of Nod factors have been performed to study structure-function relationships. Removal of the O-acetate group results in a slight decrease in the ability to form nodules [13, 16]. Each of the following modi-
flications, reduction of the anomeric carbon of the reducing sugar, removal of the sulphate group, or hydrogenation of the double bonds of the N-acyl chain results in a strong decrease in the ability to induce nodules [16]. These results show that the anomeric carbon, the sulphate group and at least one of the double bonds of the fatty acid chain are essential for organogenetic activity on lucerne.

The host range nodH and nodPQ genes determine the sulphation of Nod factors

It has been proposed that the common nodABC genes determine the synthesis of a Nod factor precursor(s) and that the function of the host-specific nod genes is to mediate the modification of this precursor(s) to generate plant-specific signals [11, 18]. The role that individual nod genes play in the synthesis of the Nod factors is now subject to much attention. The nodH and nodPQ genes are the major host range genes of *R. meliloti* and have been shown to control the specificity of the Nod factors [11, 18]. *NodH* mutants produce Nod factor molecules identical to those produced by the wild type except that they are not sulphated [13]. *NodP* and *nodQ* mutants produce a mixture of sulphated and non-sulphated factors. This partial sulphation of the lipooligosaccharides is probably caused by the presence of a functional reiteration of the *nodPQ* genes [19, 20], since strains which carry mutations in the two *nodPQ* copies do not produce sulphated factors [13]. Thus both *nodH* and *nodPQ* genes are involved in the sulphation of the Nod factors. The *nodP* and *nodQ* genes are homologous to *Escherichia coli* cysD, cysN and cysC genes and they encode ATP sulphurylase and APS kinase [20, 21]. They are responsible for the production of an activated form of sulphate, 3'-phosphoadenosine, 5'-phosphosulphate (PAPS) [20, 21]. The *nodH* product, homologous to sulphotransferases, is likely to transfer sulphate from PAPS to the Nod lipooligosaccharide precursors [13].

Purified sulphate factors from *R. meliloti* elicit root hair deformations, cortical cell division and nodule formation on lucerne but are not active on vetch, a non-homologous host of *R. meliloti* [13]. In contrast, the non-sulphated compounds produced by the *nodH* and *nodPQ* mutants elicit root hair deformations and root shortening on vetch and are not active on lucerne. There is thus a correlation between the symbiotic specificity of the various *R. meliloti* bacterial strains and the specificity of the activity of purified Nod factors [13]. These results allow us to propose the hypothesis that *Rhizobium* determines host specificity, infection and nodulation through the production of the lipo-oligosaccharide Nod factors.

Conclusion

Future work on the bacterial side will analyse the role of each individual *R. meliloti* nod gene in the synthesis and transport of the Nod factors by (i) determining the structure of the factors which accumulate in each nod mutant, (ii) exploiting the homologies of the putative nod gene products with known proteins, and (iii) physiological and biochemical studies. On the plant side, the availability of signal molecules which elicit a genuine organogenesis, and which can be modified at will by genetic or chemical means, will allow the study of how a signal is perceived, transduced and elicits a major developmental switch in the plant.

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be secreted rather than cell retained. In both frac-


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**Sulphated glycoconjugates in the immune system**

Christopher C. Rider
Department of Biochemistry, Royal Holloway and Bedford New College, University of London, Egham Hill, Egham, Surrey TW20 0EX, U.K.

**Proteoglycans and glycosaminoglycans**

Proteoglycans and glycosaminoglycans are conventionally thought of as constituents of the extracellular matrix of connective tissue, where they are indeed abundant. However, it is now emerging that probably all cell types synthesize sulphated proteoglycans, a complex process of multiple biosynthetic stages. Lymphocytes, even in the resting state, synthesize chondroitin sulphate and heparan sulphate proteoglycans, a complex process of multiple biosynthetic stages. 1-4.

Despite the fact that all the lymphocyte surface proteins and secreted proteins active in the immune system are glycosylated polypeptides, comparatively little attention has been paid to lymphocyte proteoglycans.

In our studies we have demonstrated that murine T lymphocytes synthesize both chondroitin sulphate and heparan sulphate proteoglycans [5]. In unstimulated splenic T cells, metabolically labelled with [35S] sulphate for 4 h, slightly greater amounts of the resulting labelled proteoglycans are found to be secreted rather than cell retained. In both fractions there is approximately equal incorporation into heparan and chondroitin sulphates [5]. The murine T lymphoma lines RDM-4 and EL-4 tend to show higher rates of incorporation; more secretion rather than retention of the incorporated label; and, the synthesis of a higher proportion of heparan sulphate to chondroitin sulphate [5]. Human peripheral blood T cells, expanded in vitro with interleukin 2 and phytohaemagglutinin, have been shown elsewhere to synthesize only chondroitin sulphate proteoglycans [4], and at present it is unclear whether the absence of heparan sulphate in these experiments arises from a species difference; use of peripheral blood rather than splenic T cells; or, a difference between resting and activated cells.

It is well established that the synthesis of sulphated glycosaminoglycans by lymphocytes is markedly increased on mitogenic stimulation [1–3]. For example, mouse splenic T cells incubated with 2–4 μg/ml concanavalin A showed a 3-fold increase in the sulphation of cellular glycosaminoglycans and an approximately 20-fold increase in the sulphation of secreted glycosaminoglycans [6]. As these increases may be detected within 24 h of mitogen exposure, changes in glycosaminoglycan biosynthesis appear to be early events in the activation of lymphocytes.

There are also qualitative differences in the chondroitin sulphate synthesized by various lymphocyte types. For instance, resting thymocytes produce exclusively 4-O-sulphated chondroitins but on concanavalin A exposure there is a switch to the production of both the 6-O- and 4-O-sulphates [1]. Similarly murine T lymphocytes synthesize almost exclusively the 4-O-sulphate, whereas B lymphocytes synthesize a preponderance of the 6-O-sulphate [7]. These lineage-specific differences are maintained in the murine B lymphoma AKTb-1b and the thymoma EL-4 [7]. These results suggest that glycosaminoglycan biosynthesis in lymphocytes is subject to complex regulation. Changes in the position of the bulky and highly

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Abbreviations used: MHC, major histocompatibility complex; FGF, fibroblast growth factor; IL, interleukin; GM-CSF, granulocyte-macrophage colony stimulating factor.