Molecular Interactions between *Rhizobium* and Legumes

Peter Skorpil, William J. Broughton

1 Introduction

Nitrogen availability limits the biosynthesis of proteins, amino acids, nucleotides and vitamins in plants more than any other element. Plants assimilate nitrogen as nitrate, and modern agriculture is highly dependent on nitrogenous fertiliser at a global annual cost exceeding 300 million $ US. Production of nitrogenous fertilisers requires huge energy inputs, and leaching of nitrate causes environmental problems such as contamination of ground water and surface streams (Gresshoff 2003). More than a century ago, Hellriegel and Wilfarth (1888) identified rhizobia as a source of nitrogen fixation. Bacterial nitrogen fixation contributes approximately the same amount of nitrate to agriculture as the application of chemical fertilisers, yet it is free of charge and without adverse environmental effects (Gage 2004). Gram-negative soil bacteria, collectively called rhizobia, induce the formation of nodules on many, but not all, leguminous plants [e.g., soybeans (*Glycine max*), common beans (*Phaseolus vulgaris*), peas (*Pisum sativum*), alfalfa (*Medicago sativa*), etc] and one non-legume, *Parasponia andersonii* of the elm family (*Ulmaceae*). Another micro-symbiont, *Frankia* spp. (Actinomycetales), nodulates and fixes nitrogen in eight different families of flowering plants including the *Betulaceae, Casuarinaceae, Coriariaceae, Dastiscaceae, Elaeagnaceae, Myricaceae, Rhamnaceae,* and *Rosaceae* (Hirsch et al. 2001). Nevertheless, due to the importance of legumes in modern agriculture, and since rhizobia are much easier to cultivate than frankia, much of our understanding of biological nitrogen fixation (BNF) in plants derives from research on legume-*Rhizobium* associations. Accordingly, this review will be concentrated on a few selected aspects of the *Rhizobium*-legume symbioses such as bacterial symbiotic signals (Nod-factors, surface polysaccharides and secreted proteins) and the plants' perception of Nod-factors.

P. Skorpil, W.J. Broughton (e-mail: william.broughton@bioveg.unige.ch)
Laboratoire de Biologie Moléculaire des Plantes Supérieures (LBMPS), Sciences III, Université de Genève, 1212 Genève 4, Switzerland

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2 Nod-Factors

Plants initiate the molecular dialogue with rhizobia by releasing flavonoids into their rhizospheres (Schultze and Kondorosi 1998; Broughton et al. 2003). These flavonoids are then taken up by the bacteria where they bind NodD proteins of the LysR family of transcriptional regulators (Broughton et al. 2000). The promoters of genes relevant for Nod-factor synthesis (*nol*, *noe* and *nod* genes) contain conserved 49 bp motifs called *nod*-boxes (Feng et al. 2003). NodD proteins bind *nod*-boxes as tetramers even in the absence of flavonoids, but activate *nod*-box controlled genes only in the presence of flavonoids (Fisher and Long 1993). This way, the timing and levels of production of Nod-factors are carefully controlled (Kobayashi et al. 2004) since overproduction of Nod-factors may provoke plant defence reactions and lead to the abortion of the infection process (Savouré et al. 1997; Hogg et al. 2002; Ramu et al. 2002). Regulation of Nod-factor production has been well studied in *Rhizobium* sp. NGR234 (from now on referred to as NGR234) (Fellay et al. 1995; Perret et al. 1999; Kobayashi et al. 2004). The symbiotic plasmid of NGR234 (pNGR234*a*) contains 19 *nod*-boxes, 18 of which are functional. Upon binding to flavonoids, NodD1 activates transcription of the genes required for Nod-factor synthesis (*nod*-genes). NodD1 also activates transcription of the *ttsI* and *syrM*2 genes. TtsI is a transcriptional regulator that activates genes controlled by *tts*-boxes, leading to the induction of the type three secretion system (T3SS) (Marie et al. 2004) (Section 8.5 and Fig. 1). In turn SyrM2, another transcriptional activator, modulates transcription of the *nodD*2 gene. In concert with TtsI, NodD2 also up-regulates transcription of genes required for the synthesis of rhamnose-rich lipo-poly-saccharides (LPS) (Marie et al. 2004) (section 8.4) and ultimately represses NodD1. In this way, the Nod-factor regulatory circuit is eventually closed (Kobayashi et al. 2004). Thus, flavonoids induce the production of Nod-factors, which are the first bacterial signals perceived by the plant and are crucial to nodulation (Relić et al. 1994a). Nod-factors are acylated lipo-chito-oligosaccharides made of 2 to 6 β-1,4-linked *N*-acetyl-D-glucosamine units carrying a fatty acid chain at the non-reducing terminus (Perret et al. 2000). Some rare Nod-factors have a slightly different oligo-saccharide backbone (Demont et al. 1994; Bec-Ferté et al. 1996; Olsthoorn et al. 1998; Pacios-Bras et al. 2002). Chain length of the oligo-saccharide, the type of the acyl moiety, the nature of the fatty acid and various decorations resulting from fucosylation, sulphation, acetlyation, *N*-methylation, 3-, 4-, or 6-, *O*-carbamoylation, 6-*O*-glycosylation, *D*-arabinosylation or 2-*O* methylation all contribute to the great variety of Nod-factors known (Perret et al. 2000). Nod-factor structures and Nod-factor biosynthesis have been extensively reviewed (Dénarié et al. 1996; Broughton et al. 2000; Perret et al. 2000). Unfortunately, there are few