

Rhizobial and Mycorrhizal Symbioses in *Lotus japonicus* Require Lectin Nucleotide Phosphohydrolase, Which Acts Upstream of Calcium Signaling^{1[C][W][OA]}

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Nodulation in legumes requires the recognition of rhizobially made Nod factors. Genetic studies have revealed that the perception of Nod factors involves LysM domain receptor-like kinases, while biochemical approaches have identified LECTIN NUCLEOTIDE PHOSPHOHYDROLASE (LNP) as a Nod factor-binding protein. Here, we show that antisense inhibition of *LNP* blocks nodulation in *Lotus japonicus*. This absence of nodulation was due to a defect in Nod factor signaling based on the observations that the early nodulation gene *NODULE INCEPTION* was not induced and that both Nod factor-induced perinuclear calcium spiking and calcium influx at the root hair tip were blocked. However, Nod factor did induce root hair deformation in the *LNP* antisense lines. *LNP* is also required for infection by the mycorrhizal fungus *Glomus intraradices*, suggesting that *LNP* plays a role in the common signaling pathway shared by the rhizobial and mycorrhizal symbioses. Taken together, these observations indicate that *LNP* acts at a novel position in the early stages of symbiosis signaling. We propose that *LNP* functions at the earliest stage of the common nodulation and mycorrhization symbiosis signaling pathway downstream of the Nod factor receptors; it may act either by influencing signaling via changes in external nucleotides or in conjunction with the LysM receptor-like kinases for recognition of Nod factor.

The acquisition of mineral nutrients from the environment often limits plant growth, and many plants have established symbiotic interactions with beneficial microorganisms that facilitate nutrient acquisition. The association with arbuscular mycorrhizal fungi helps in

nutrient uptake, particularly phosphate, and this symbiosis is almost ubiquitous within the plant kingdom, reflecting its early establishment during the evolution of higher plants (Parniske, 2008). Several plant species also form symbioses with nitrogen-fixing bacteria such as rhizobia and *Frankia* spp., and these symbioses are restricted to plants in the Rosid I clade (Doyle, 1998). The establishment of both mycorrhizal and rhizobial interactions in legumes involves a molecular signal exchange between the plant and its symbiont. Legumes release strigolactones and flavonoids into the rhizosphere, and these are recognized by mycorrhizal fungi and rhizobia, respectively (Oldroyd et al., 2009). In turn, the symbionts release signals to the plant: lipochitoooligosaccharide Nod factors from rhizobia (Dénarié et al., 1996) and Myc factors from mycorrhizal fungi (Kosuta et al., 2003; Oláh et al., 2005; Maillet et al., 2011). Species-specific decorations on the Nod factor backbone are recognized by the host legume, and this determines specificity in the legume-rhizobial interaction (Perret et al., 2000). In contrast, there appears to be little specificity in mycorrhizal interactions (Parniske, 2008).

Nodulation-defective legume mutants have revealed a signaling pathway that is conserved between Nod factor and mycorrhizal recognition (Wais et al., 2000; Walker et al., 2000; Kistner et al., 2005), and considering the different evolutionary histories of these two

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