Trends in Plant Science

CellPress

Special Issue: Unravelling the Secrets of the Rhizosphere

Opinion

Into the Root: How Cytokinin Controls Rhizobial Infection

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Leguminous plants selectively initiate primary responses to rhizobial nodulation factors (NF) that ultimately lead to symbiotic root nodule formation. Functioning downstream, cytokinin has emerged as the key endogenous plant signal for nodule differentiation, but its role in mediating rhizobial entry into the root remains obscure. Nonetheless, such a role is suggested by aberrant infection phenotypes of plant mutants with defects in cytokinin signaling. We postulate that cytokinin participates in orchestrating signaling events that promote rhizobial colonization of the root cortex and limit the extent of subsequent infection at the root epidermis, thus maintaining homeostasis of the symbiotic interaction. We further argue that cytokinin signaling must have been crucial during the evolution of plant cell predisposition for rhizobial colonization.

Trends

Cytokinin regulates many aspects of plant development, including symbiotic root nodule formation in leguminous plants.

The role of cytokinin as the endogenous plant inducer of nodule primordia formation has now been firmly established.

Current data implicate cytokinin in the regulation of root colonization by nitrogen-fixing bacteria, but the underlying mechanism remains unclear.

Plant mutants with defects in cytokinin signaling (receptors) show aberrant rhizobial infection phenotypes.

Nitrogen-Fixing Symbiosis

Approximately 60 million years ago, evolutionary events, as yet not fully understood, gave rise to a developmental novelty, the symbiotically derived root nodule organ [1,2]. Nodules host nitrogen-fixing rhizobial bacteria in intracellular, organelle-like structures called '**symbiosomes**' (see Glossary), providing an environment conducive for the bacterially encoded **nitrogenase** to carry out the reduction of dinitrogen to ammonia. This endosymbiotic liaison of a selected group of plants with soil bacteria [3] renders the host capable of tapping into the vast reservoir of nitrogen stored in the atmosphere of the Earth, effectively making its growth independent from soil nitrogen [4]. Symbiotic nitrogen fixation is of significant importance, especially considering current challenges associated with the cost and negative environmental impact of anthropogenic nitrogen [5].

Symbiosis and Cytokinin Signaling Pathway

Root nodule organogenesis is triggered by rhizobially produced lipochito-oligosaccharide molecules, known as NF [6]. The perception of NF by the host plant LysM motif receptor kinase complex [7] initiates a range of primary cellular responses, including the root hair tip-localized calcium (Ca^{2+}) influx and Ca^{2+} spiking [8]. Acting as a secondary messenger, the NF-induced Ca^{2+} evolutions facilitate links with intrinsic plant developmental pathways. This is achieved, at least in part, through the regulation of CALCIUM and CALMODULIN-DEPENDENT RECEPTOR KINASE (CCaMK) [9–11]. The resultant signaling outputs lead to activation of several transcription regulators, including CYCLOPS, a protein that interacts with CCaMK [12] and the NODULE INCEPTION (NIN) activator [13]. These and other regulators partake in the transcriptional reprograming [14] that mediates the **infection thread** (IT)-dependent entrance of rhizobia inside the root [15] and also stimulates a subset of susceptible cells to form a nodule primordium within the subtending root cortex [4,6].

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Key Figure

A Conceptual Model for Cytokinin-Dependent Regulation of *Mesorhizobium loti* Infection in *Lotus japonicus*

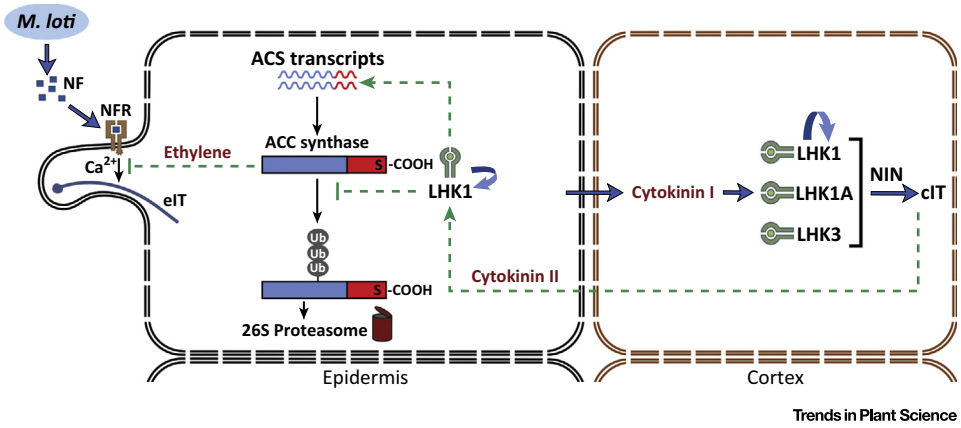

Figure 1. *Mesorhizobium loti* infection and perception of nodulation factor (NF) by the NF receptor complex (NFR) generate a presumed cell nonautonomous signaling event (the blue arrow that crosses an epidermis–cortex boundary), triggering the first cytokinin activity peak within the root cortex (Cytokinin I). As perceived by LOTUS HISTIDINE KINASE (LHK) cytokinin receptors and in a NODULE INCEPTION (NIN)-cortex-dependent manner, the permissive conditions for cortical infection thread (cIT) formation are generated. The same signaling events contribute to a negative feedback loop, which leads to the second cytokinin activity peak in the root epidermis Cytokinin II); this is presumed to locally block subsequent epidermal infections (eIT). Cytokinin might increase the level of ethylene production in a LHK1-dependent manner by enhancing the steady-state level of mRNA encoding 1-aminocyclopropane-1-carboxylic acid synthase (ACS) transcripts and/or by increasing the stability of the type-2 ACS proteins, preventing their degradation via a ubiquitin-proteasome pathway. Ethylene blocks Ca^{2+} signaling, which is required for eIT formation. The 'u-turn' arrows denote autoactivation of *LHK1* gene expression, which is alleged to increase the sensitivity of cells to cytokinin. Positive and negative regulatory actions are indicated by arrows and lines with bars, respectively.

Artificial activation of several components that participate in the symbiotic signaling, including NF receptors, CCaMK or CYCLOPS, induces the formation of empty nodules independent of ***Rhizobium*** and/or NF [12,16–19]. Ectopic **cytokinin** is also sufficient to stimulate nodule structure formation on at least some leguminous roots [20,21]. It has become clear that cytokinin signaling, induced downstream from the NF perception and CCaMK, represents one of the key endogenous effectors of nodule differentiation [20–28]. In *Lotus japonicus*, this process is mediated by the Lotus Histidine Kinase 1 (LHK1) cytokinin receptor with a partially redundant involvement of LHK1a and LHK3, requiring several cytokinin-regulated effectors, including NIN and Nodulation Signaling Pathway 2 (NSP2) [13,20,23,24,29,30]. Importantly, in both *lhk1-1* and the *lhk1-1 lhk1a-1 lhk3-1* triple receptor mutant, defects in nodule formation are accompanied by hyperinfection of the root epidermis by *Mesorhizobium loti* [23,27]. This implies that cytokinin signaling, as mediated by LHK1 and possibly other cytokinin receptors, is not only essential for nodule structure formation, but also partakes, directly or indirectly, in establishing homeostasis of the symbiotic infection. Neither of the *lhk1a-1* and *lhk3-1* single mutants nor the corresponding *lhk1a-1 lhk3-1* double mutant are hyperinfected by *M. loti*, which indicates that, in *L. japonicus*, LHK1 is not only required for, but also sufficient to restrict the epidermal infection thread (eIT) formation events (Figure 1, Key Figure).

Into the Root Epidermis

In most legumes, including *L. japonicus* and *Medicago truncatula*, the primary mode of rhizobial entry inside the root is by root hair eITs [15]. Only a small proportion of eITs will enter the

Glossary

1-Aminocyclopropane-1-Carboxylic Acid (ACC): a precursor for ethylene that is synthesized by the enzyme ACC synthase from the amino acid methionine.
ACC deaminase: enzyme that cleaves ACC into ammonia and α-ketobutyrate, thus decreasing ACC levels. This multimeric enzyme is a common component of many soil microorganisms.
ACC synthase (ACS): enzyme that catalyzes the synthesis of ACC, a precursor for ethylene, from *S*-adenosylmethionine (*Ado-met*).
Autoregulation of nodulation (AON): a genetic mechanism that dynamically limits the number of nodules through a systemic, root to shoot to root long-distance signaling feedback loop.
Bacteroid: a symbiotic form of rhizobia that reside within the plant cells and fix atmospheric nitrogen into ammonium.
Calcium spiking: transient, repetitive, increases in calcium levels in and around the nucleus of the responding cells.
Cytokinin: an adenine-derived hormone involved in regulation of diverse plant developmental processes.
Determinate nodules: usually of a round shape, lack an obvious developmental gradient, and are deprived of a persistent meristem; examples include soybean (*Glycine max*) and *Lotus japonicus*.
Ethylene: a gaseous plant hormone that has an important role in many growth and developmental processes.
Indeterminate nodules: usually of a rod-like shape with a well-defined developmental zonation and a persistent meristem; examples include pea (*Pisum sativum*) and *Medicago truncatula*.
Infection threads (ITs): plant plasma membrane-derived conduit by which rhizobia enter and colonize plant roots.
Nitrogenase: a prokaryotic metalloenzyme complex that catalyzes the reduction of dinitrogen to ammonia.
Pre-infection thread: a column of aligned cytoplasmic bridges within the activated root cortex through which infection threads propagate.