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Same but different: examining the molecular mechanisms of intercellular rhizobial infection

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Mutually beneficial symbioses between plants and microbes are widespread and likely arose shortly after plants began to grow on land (Martin et al., 2017). In exchange for photosynthate, microbial symbionts often provide nutrients that would otherwise be inaccessible to plant roots. Legume plants of the Fabaceae family form special nodules in their roots to harbor nitrogen-fixing rhizobia bacteria. Due to the importance of nitrogen in plant growth, and because the Fabaceae family contains many important crop species, the biology of this rhizobial symbiosis has been a focus of study, and many signaling components have been uncovered. However, much of this research has been carried out in a few model legumes together with their native symbionts. Different patterns of legume–rhizobia symbioses have also been reported in nonmodel legumes, but since genetic tools for these species are lacking, it is unclear whether these alternative symbioses require the same molecular components as the models.

In this issue of *Plant Physiology*, Montiel et al. (2021) show that the model legume *Lotus japonicus* can successfully establish a symbiosis with a rhizobial strain that follows a different infection strategy from the native *L. japonicus* symbiont. Taking advantage of the genetic resources available for *L. japonicus*, they show that this alternative symbiosis involves many of the known symbiosis-associated molecular components, but there are also differences. The work of Montiel et al. (2021) helps to shed light on the evolutionary history of legume–rhizobia symbioses.

Legume roots perceive compounds called Nod factors, produced by compatible rhizobia in the soil. In model legume–rhizobia systems such as *L. japonicus* and its symbiont *Mesorhizobium loti*, the bacteria enter the root through an infection thread, a tube of plasma membrane that forms at the tip of a root hair and extends through the root hair cell into the cortex (Figure 1A). However, some legumes do not form infection threads, and the rhizobia enter the root between intact epidermal cells. Such colonization without a root hair infection thread is termed intercellular infection. To study the molecular events that occur during intercellular colonization, Montiel et al. (2021) inoculated *L. japonicus* with the rhizobial strain IRBG74, which intercellularly infects its native host legume *Sesbania cannabina* under flooded conditions (Goormachtig et al., 2004). Interestingly, *L. japonicus* formed functional nodules with IRBG74, albeit less rapidly than with its native symbiont. Microscopy confirmed that root hair infection threads do not form in response to IRBG74; rather, the bacteria accumulate on the surface of the root and enter the root intercellularly. Once inside the root, infection of the nodule occurs via plasma membrane invaginations, which form infection pegs or transcellular infection threads (Figure 1).

The authors then tested how intercellular IRBG74 infection was affected in *L. japonicus* mutants that are defective in symbiosis with *M. loti*. Nodulation with IRBG74 was reduced or abolished in most of these mutants, indicating that a conserved symbiosis signaling pathway is activated by both symbionts. Some of the mutated genes are required to trigger formation of the nodules themselves, by stimulating cell division within the cortex, and thus the lack of nodulation is not surprising. However, a number of the mutants are more specific to infection thread formation. For example, when the cytoskeletal component *SUPPRESSOR OF cAMP RECEPTOR DEFECT—NODULATION* (*ScarN*) is mutated, nodules form on the roots but do not become populated by rhizobia (Qiu et al., 2015). Interestingly, this phenotype was also observed with IRBG74 (Montiel et al., 2021).

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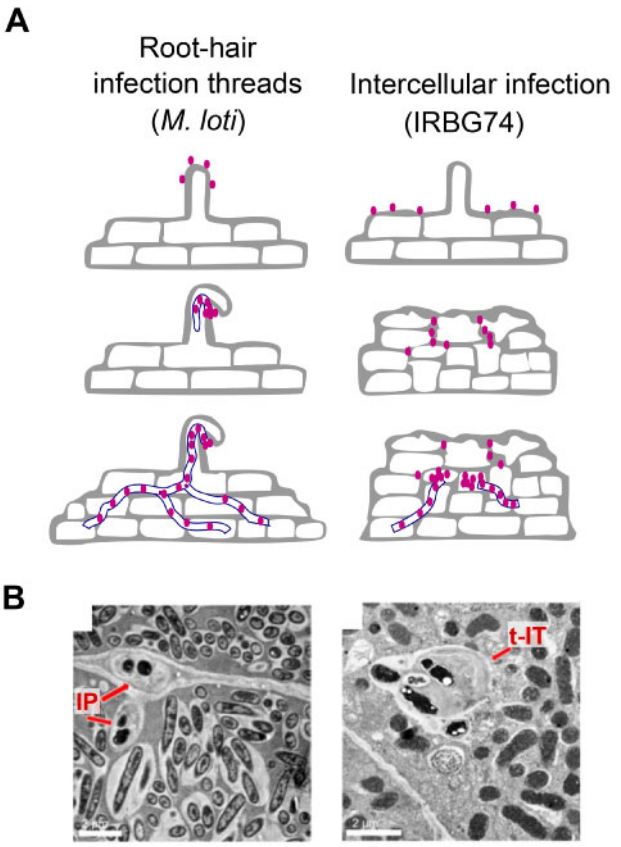

Figure 1 Rhizobia IRBG74 infects *L. japonicus* intercellularly. A, Diagrams of root infection strategies. The native *L. japonicus* symbiont *M. loti* enters the root through an intracellular root hair infection thread, which also spreads the bacteria to the cortical cells of the developing nodule. In contrast, rhizobia IGBF74 enters the root between epidermal cells and forms subepidermal infection pockets. Infection pegs and transcellular infection threads then distribute the bacteria to the cortical cells. B, Transmission electron micrographs showing examples of IRBG74 infecting cortical cells via infection pegs (IPs) or transcellular infection threads (t-ITs). Figure modified from Montiel et al. (2021) by Charles Copeland.

Colonization of the nodules after intercellular root infection, therefore, seems to require similar cytoskeleton remodeling processes as the formation of root hair infection threads.

Although intercellular infection with IGBF74 does depend on many of the conserved symbiosis-associated genes, Montiel et al. (2021) also found some differential requirements. The transcription factor ETHYLENE RESPONSE FACTOR REQUIRED FOR NODULATION 1 (ERN1), like ScarN, is required for infection thread formation, and *ern1* mutants inoculated with *M. loti* produce fewer infected nodules, arising via intercellular infection (Kawaharada et al., 2017). In contrast, nodulation in *ern1* plants inoculated with IGBF74 is comparable to wild-type plants. Although no infection threads are observed in the *ern1* mutant, infection pegs do form, and appear to be sufficient for normal nodule infection.

To further characterize the differences in signaling during intercellular infection, the authors analyzed transcriptional changes using RNA-seq. While most of the conserved symbiosis genes were induced by both rhizobial strains, the overall transcriptional changes were otherwise quite different. Several genes related to cytokinin signaling are differentially regulated by infection with IGBF74 compared to *M. loti*. By testing mutants in some of these genes, the authors found that the two infection methods involve cytokinin signaling mediated through different genes. It is likely that intercellular infection by IGBF74 involves further distinct host signaling components that remain to be identified.

The bacterial factors responsible for intercellular infection are also unknown. The actual mechanism of entry into the root is likely not legume–specific, as IGBF74 can also colonize rice (*Oryza sativa*) roots (Mitra et al., 2016). Besides Nod factor, IGBF74 may also produce other chemical cues required for nodule formation and infection. The availability of mutants and molecular tools for *L. japonicus* will facilitate discovery of the molecular players in both the host and bacteria.

Knowledge of the genetic requirements for different plant-microbe symbioses is crucial to resolve the evolutionary history of these associations. It is thought that a predisposition for nodulation arose through the recruitment of the signaling pathway already in use for symbiosis with arbuscular mycorrhizal fungi (Martin et al., 2017). Subsequent gains and losses in N-fixing symbiosis then led to diversity of legume–rhizobia associations found today. Intercellular infection is thought to be the evolutionarily basal mechanism (Ibáñez et al., 2017). It seems likely that less-derived symbioses would have taken advantage of Rhizobiales bacterial strains that were already present in the root microbiota (Garrido-Oter et al., 2018), and that strategies to recruit specific bacterial strains evolved later. These basal plant-rhizobia interactions may also require fewer signaling components, and would therefore be the logical starting point in an attempt to engineer nitrogen fixation in nonlegume crops (Ibáñez et al., 2017). However, the work of Montiel et al. (2021) demonstrates that intercellular infection is not necessarily a simple legume–rhizobia symbiosis mechanism; rather, it involves most of the conserved symbiosis-associated genes as well as its own distinct signaling components.

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