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Transcription factors link rhizobia, legumes

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Scientists have identified two putative plant transcription factors that are essential links in the symbiosis of rhizobial bacteria and legumes, according to two reports in this week's *Science*. The transcription factors—GRAS family proteins NSP1 and NSP2—may play distinct yet cooperative roles in regulating the development of nodules on the plant roots where the nitrogen-fixing bacteria reside.

"The interesting aspect of this is that we have a couple of GRAS proteins here that are fairly well positioned in the signaling pathway with clear phenotypic effects," Michael Udvardi, of the Max Planck Institute of Molecular Plant Physiology, Golm, Germany, and coauthor of a *Perspective* accompanying the studies, told *The Scientist*.

Legumes initiate the symbiotic relationship between bacteria and plant by emitting flavonoid compounds that are recognized by the bacteria. Rhizobia then produce *Nod factors*, oligosaccharides that elicit dramatic alterations in the gene expression and metabolism of both organisms. In the past few years, scientists have identified genes for "the small set of key regulators"—including a *Nod factor receptor* and *possible plastid cation channels*—in the initial steps of symbiosis, according to Ton Bisseling at Wageningen University, the Netherlands, coauthor of *one of the papers*. But for the first time, researchers have identified players at the first step that is specific to the rhizobia/legume interaction—what the authors of the *second paper* call Nod factor response factors.

Previous studies had demonstrated that *nsp1* and *nsp2* mutants were unable to produce the burst of gene expression that usually follows Nod factor stimulation and calcium spiking in epidermal root cells. The two teams used map-based cloning in the model legume *Medicago truncatula* to identify genes encoding the putative transcription factors NSP1 and NSP2.

According to the reports, the findings were consistent with earlier evidence that related proteins in the GRAS family are known to regulate transcription in other plants and contain structural elements—including leucine-rich regions and homopolymeric stretches in the N-terminal region—often found in transcriptional regulators.

Using green fluorescence protein tagging, the authors found that NSP1 and NSP2 localized to the nucleus, where they compensated for the nodulation defects in *nsp1* and *nsp2* mutants. That placed the proteins near the nuclear calcium/calmodulin-dependent protein kinase (CCaMK), which is known to act upstream, and to potential DNA targets. The authors conclude that this localization implies roles in calcium transduction for NSP1, which is constitutively active, and for NSP2, which shifts to the nucleus from the nuclear envelope.

It will now be important to look for "interactions between key regulators," such as the genes "directly activated by NSP1 and 2" and the potential dimer interaction of the proteins, Bisseling said in an E-mail. According to Udvardi, the next steps in this research will also involve "deciphering the subsequent changes" in the plant's gene expression, which he said "is more difficult genetically because of pleiotropic effects downstream." The next wave of genetic tools—functional genomics approaches such as DNA profiling assays—will be crucial to complement classic methods, he said.

Udvardi said the implications of the studies may go [beyond legumes](#). Though legumes are the only plants that use rhizobia for their nitrogen fixing needs, 90% of land plants have been using part of the same signaling pathway for the past 460 million years to get help from arbuscular mycorrhizal fungi in obtaining phosphate and water from the soil, he said. And less than 20 million years ago, plant nematodes appropriated the same pathway elements to invade legumes.

According to [David Bird](#) of North Carolina State's Center for the Biology of Nematode Parasitism, who did not participate in this study, a [paper he coauthored earlier this year](#) suggests that these parasites may even have acquired genes directly from Rhizobia through horizontal transfer. The imminent completion of model legume genome sequencing projects—by this year or the next, according to Bird—mean that discoveries made here could quickly be transferable to other plant species, Udvardi said.

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