

Title: Dynamic change in chromatin accessibility predicts regulators of nodulation in *Medicago truncatula*

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Project Goals: Our research goal is to identify host genomic elements involved in the symbiotic relationship between legume roots and nitrogen (N₂)-fixing rhizobium bacteria. In order to identify such elements, we measured and analyzed novel RNA-seq and ATAC-seq time-course data obtained from *Medicago truncatula* roots subjected to treatment with the rhizobial symbiotic signals, lipo-chitoooligosaccharides (LCO). The gene regulatory network involved in the response to LCOs was predicted by using a novel computational method that defines dynamically transitioning genes and predicts key regulators of these genes. Prioritized regulators and their target genes are now validated experimentally with RNAi experiments. The RNAi results reveal that *Ethylene Insensitive 3 (EIN3)* and *Ethylene Response Factor 1 (ERF1)* are essential for rhizobium-legume symbiosis.

Abstract Text: Rhizobia can establish symbiotic associations with legume roots resulting in the formation of root organs called nodules. This symbiosis triggers extensive genome and transcriptome remodeling in the host plant, yet the extent of chromatin changes and impact on gene expression is not well known. We profiled the chromatin accessibility (ATAC-seq) and transcriptome (RNA-seq) dynamics of *M. truncatula* roots treated with rhizobial LCOs over a 24 hr period (0 (control), 15 and 30 min, and 1, 2, 4, 8, and 24 hours).

Using a novel approach, Dynamic Regulatory Module Networks,¹ we predicted gene expression as a function of chromatin accessibility and accessible *cis*-regulatory elements within 10 kbp upstream to 1 kbp downstream of gene transcription start sites. This approach identified the *cis*-regulatory elements and associated transcription factors that most significantly contribute to transcriptomic changes triggered by LCOs. Regulators involved in auxin (IAA4-5, SHY2), ethylene (EIN3, ERF1) and abscisic acid (*ABI5*) hormone response, as well as histone and DNA methylation (IBM1), emerged among those most predictive of transcriptome dynamics.

We validated our prioritized set of regulators by knocking down regulators with RNAi and measuring the number of nodules in mutant and wild type plants. The knockdown of *EIN3* and *ERF1* was found to reduce the nodule number in *M. truncatula* roots relative to the empty vector control suggesting that these regulators are important for nodulation. These findings are corroborated by previous results in *L. japonicus*.² The molecular mechanisms by which *EIN3* and *ERF1* regulate rhizobium-legume symbiosis remain to be explored. Taken together, our dataset and results provide novel insights into the regulation of gene expression in LCO induced responses in legumes and should be useful to the broad community of researchers interested in mechanisms underlying symbiotic relationships between plants and microbes.

References/Publications

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