PhD project

Functional study of a long non-coding RNA involved in the Rhizobial and the Arbuscular Mycorrhizal Symbioses

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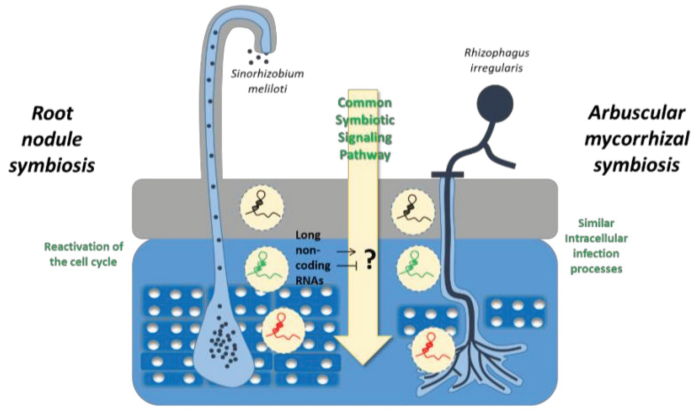
Symbiotic interactions between legumes and rhizobial nitrogen-fixing bacteria, and many plants and arbuscular mycorrhizal fungi are of high agricultural and ecological value. When legumes interact with rhizobia, a new organ called a nodule forms on the root. These nodules provide a favorable environment for bacterial fixation of atmospheric nitrogen. In contrast, arbuscular mycorrhizal symbiosis (AMS) leads to fungal colonization in the inner cortex of the root, and the formation of intracellular arbuscules allowing nutritional exchanges between the fungus and the plant. Genetic studies have shown that both bacterial and fungal symbioses share a common signal transduction pathway (Oldroyd, 2013). This common symbiotic pathway, would act downstream of the perception of fungal and rhizobial signals and upstream of the activation of the appropriate response to each symbiont. Despite the differences between the two types of symbiosis, similarities also exist downstream of this common genetic pathway, including mechanisms of symbiotic infection preparation and cell cycle activation.

In recent years, long non-coding RNAs (lncRNAs) that are genes that lack protein-coding potential, have been described as a new class of regulators playing a role in a wide range of cellular regulatory mechanisms, including the control of gene expression *via* chromatin modifications (Fonouni-Fardes et al., 2021). It has been shown recently that a large proportion of the genes regulated during the legume-rhizobium symbiosis are clustered on the genome of the model legume *Medicago truncatula* in genomic regions called "symbiotic islands" (Pecrix et al., 2018). These symbiotic islands are genomic regions averaging 40 kb that contain genes co-regulated during rhizobial symbiosis and that are strongly enriched in lncRNAs. Consequently, a role in the coregulation of genes present on symbiotic islands has been proposed for these lncRNAs. We have recently identified the existence of symbiotic islands containing genes activated during AMS. On one of these “myc islands” we have identified a lncRNAs that we called *MN2* (*Myconod2*). Expression studies using RNA-seq and promoter-GUS reporter genes showed a strong and specific upregulation of *MN2* during the initiation of both symbioses. We have initiated a functional analysis of *MN2* and preliminary results using RNAi and overexpression approaches suggest a role for *MN2* as a negative regulator of nodule development.



The PhD project will be divided in 3 parts.

1) Functional analysis of *MN2* activity during both symbioses

1-1 The analysis of overexpression and RNAi constructs in transgenic roots will be carried on and extended to the AMF symbiosis.

1-2 Tnt1 transposon insertion lines have been identified and their effect on both symbioses will be analyzed

2) Effect of *MN2* on the expression of neighboring genes present on a symbiotic island.

MN2 is positioned on chromosome 7 on a 86kb symbiotic island containing 16 genes including a cluster of 9 nitrate/peptide transporter- encoding genes. LncRNAs have been shown to regulate the expression of neighboring genes (Ariel 2014, 2020). Using the lines in which *MN2* expression is modified, (Part 1), the effect of *MN2* missexpression on neighboring genes will be analyzed using q-RT-PCR (for local changes) and RNA-seq (for a genome-wide analysis).

3) Mode of action of *MN2*

LncRNAs have been shown to function among other mechanisms by interacting with specific chromatin regions or by recruiting regulatory proteins. In this part of the project we will seek to understand the mode of action of *MN2*.

3-1 Identification of proteins interacting with *MN2*

3-2 Identification of chromatin regions bound by *MN2*

These two approaches will be performed using a technique called ChIRP (Chromatin Isolation by RNA Purification) that can be followed by the identification either of the chromatin regions bound by *MN2* by Illumina sequencing (ChIRP-seq) or of interacting proteins using a mass spectrometry approach (ChIRP-MS).

3-3 Identification of chromatin loops

lncRNAs have been shown to regulate the expression of neighboring genes *via* chromatin loops. We will identify putative *MN2*-based chromatin loops using Chromatin conformation capture (3C).

References

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