Identification and Expression Analysis of Host Genes Responsible for the Establishment of Gunnera-Nostoc Symbiosis

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Introduction

The problem of resource acquisition is one of the greatest ecological constraints on any organism. Plants, while generally able to supply themselves with sugars via photosynthesis, depend upon their substrates for many other kinds of nutrients. Nitrogen-rich fertilizers are used extensively to supply crop plants with nitrogen, resulting in a high cost to both our economy and our environment (Serhal, et al., 2009). Some plants, however, are able to generate their own supplies of nitrogen through symbioses with nitrogen-fixing microorganisms. The best-known groups are the legume family (Fabaceae) and actinorhizal plants. These plants acquire their nitrogen through symbioses with nitrogen-fixing *Rhizobia* and *Frankia* bacteria, respectively (Hirsch, 1992).

While the legumes, due to their agricultural utility, are the most famous case of plant-microbial symbiosis, other plant groups have developed novel ways of accomplishing the same goal, including symbioses with nitrogen-fixing cyanobacteria. Cyanobacterial-plant mutualism exist within every major clade of land plants (Adams, et al. 2006), including several species of liverworts and hornworts, the fern *Azolla*, cycads, and the genus *Gunnera* (Gunneraceae) in the angiosperms. *Gunnera* is a subtropical genus found throughout the southern hemisphere (Fuller and Hickey, 2005), and it is the only genus of angiosperms known to engage in symbiosis with cyanobacteria. Additionally, it is the only known case of intracellular hosting of cyanobacteria in plants (Silvester & McNamara, 1976). *Gunnera* engages in mutualism mainly with cyanobacteria in the genus *Nostoc* through a conspicuous mucilage-secreting gland that serves as the site of bacterial entrance and colonization.

In *Gunnera manicata* the gland is formed from undifferentiated mitotic cells that are located on the stem beneath the petiole. They form independently of the presence of *Nostoc* and have a very specialized morphology, including several papillae (grooves) that form around a central papilla (Chiu, 2005; Uheda & Sylvester, 2001). The channels between papillae serve as pathways for motile *Nostoc* filaments (called hormogonia) to invade the stem. Their conspicuous color is caused by the presence of anthocyanin. The mature gland also secretes a translucent mucilage prior to infection by *Nostoc* (Johansson & Bergman, 1992).

I am trying to understand how the symbiotic gland prepares itself for invasion by *Nostoc,* and, more specifically, what genes are expressed in the gland that make it attractive for hormogonia to enter and assume a symbiotic state. Because the symbiosis occurs intracellularly, there must be some alteration of the plant's natural barriers to microorganisms for entry of the symbiont to be possible. My hypothesis is that the gland does this by expressing cell wall remodeling enzymes that convert cell wall components (primarily cellulose, hemicelluloses, and pectins) into soluble sugars. This would accomplish two important functions: the breaking down of cell wall material would provide a weaker barrier to the invading bacteria, and the abundance of monosaccharides would create an enticing food source for the travel-worn hormogonia.

Progress Made During Summer 2009

To test this hypothesis, I set out to identify sequences of various cell wall hydrolases and design primers for use in quantitative reverse-transcription PCR (qRT-PCR) in order to compare the level of gene expression in the gland and neighboring stem tissue. Towards the beginning of the summer, I amalgamated a large database of genes expressed in the gland by blasting roughly 13,000 contigs obtained from 454 sequencing of a gland cDNA library against Genbank. This list was used as a reference for identifying specific gene sequences in target gene families, aiding in the formation of

primers for use in qRT-PCR. Additionally, to get a sense of what enzymatic pathways were prevalent in gland tissue, these same sequences were submitted to KEGG (Kyoto Encyclopedia of Genes and Genomes). Kegg results showed that several enzymes in our sequence data were involved in pathways related to sugar and starch metabolism, including glucan (glucose polymers linked by glycosidic bonds), sucrose, and starch degradation. (Fig 1)

Fig 1. Starch/Sucrose Metabolic pathway chart obtained from submitting our pyrosequence data to KEGG. Invertase (3.2.1.26), amylase (2.4.1.1), and various β-glucanases (3.2.1.21) are all present.

These efforts led to the identification of specific sequences for enzymes involved in degrading the major classes of cell wall polysaccharides, which could then be used to create primers for expression analysis using qRT-PCR. Because of the possibility that many different genes within a given gene family were being transcribed, and because there is often a positive correlation between transcriptional activity and 454 sequence length, I generally chose the sequences from the longest contigs obtained by 454 sequencing to construct primers for qRT-PCR.

Primers for glycoside hydrolases (cell wall remodeling enzymes) as well as related enzymes in sugar metabolism (e.g. invertase, sucrose synthase) were designed and used in qRT-PCR to compare gene expression between gland and stem tissues of the plant *G. manicata*. Certain genes, such as β-1,3-glucanase (involved in the breakdown of cell wall glucans), beta-1,4-glucosidase (terminal degradation of beta-1,4-glucans), a polygalacturonase, and pectin methylesterase (pectin modification) were consistently found to be expressed higher in the gland than in the stem. Some of these results are not conclusive, however, due to the fact that many of our results were obtained using β-tubulin as an internal control gene, which we later discarded in favor of a TIP41 family gene, whose expression has been shown to be highly consistent in different plant tissues (Rodriguez et al., 2008).

Proposal for the Academic Year

Beginning this fall I plan to assist drafting a manuscript for publication on carbohydrate metabolism in the gland. This will require me also to amplify full-length sequences of genes we find preferentially expressed in gland tissue. To do this I will create primers at the ends of each gene for regular PCR and amplify the full-length gene. I will then clone the PCR product into a plasmid. The cloned genes can be used for Sanger sequencing as well as over-expression assays.

I will also begin looking a bit broader at gene expression patterns in the Gunnera manicata gland. The transcription of many glycoside hydrolases, as well as components of various pathogenesis-related signal transduction pathways, are regulated by the Ethylene Response Factor (ERF) class of transcription factors (TFs). During the year at Oberlin, I will assist in identifying and designing qRT-PCR primers for ERFs that are expressed in the gland. I will sequence and clone ERFs that are found to be expressed at a higher level in the gland than in the stem. Subsequent over-expression assays of these TFs would test the hypothesis that the unique characteristics that make the gland an enticing destination for *Nostoc*, including the expression of the glycoside hydrolases that I studied this summer, are controlled by ERF TFs.

I also plan on taking two courses at Oberlin that are relevant to my project: Cell and Molecular Bio (fall 2009), and Bioorganic Chemistry (Spring 2010). Additionally, I plan on reading independently on plant biochemistry and signal transduction to better understand the similarities and differences between pathways involved in pathogen defense and microbial symbiosis.

Budget

To be determined

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