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Ph.D. Dissertation

“Expression patterns and genomic organization of the Proline Rich Protein family in *Medicago truncatula*”

“Investigating the role(s) of the ABI5-binding Proteins in abiotic stress tolerance in *Arabidopsis thaliana*”

**Chapter 1: Introduction for *Medicago* *truncatula* project**

Legume is a common name for angiosperms of the family Fabaceae which includes many agriculturally significant crops such as soy beans, lentils, peanuts, kidney beans, alfalfa, and garbanzo beans (chickpeas), among others. Approximately one third of the crop production on the planet is dedicated to legumes (**citation needed**) and they are an important source of dietary protein, especially in cultures that are primarily vegetarian (**citation?**). Their importance as crops was recognized millennia ago, although the exact reasoning behind their beneficial action was not realized until much more recently. One of the earliest documented acknowledgments of the agricultural importance of legumes came from Pliny the Elder in 75 A.D.: “It is universally agreed that no manure is more beneficial than a crop of lupins turned in by the plough or with forks before the plants form pods” (lupin refers to a genus within the family Fabaceae). Even today farmers practice what is known as “crop rotation,” alternating grains and grasses with legumes in the same plots to enrich the soil. Many legume species (**how many? All?**) are able to form nitrogen-fixing symbioses with soil bacteria from various genera that are collectively known as rhizobia. These symbioses occur in specialized root structures called nodules that provide an anaerobic environment which allows the conversion of inert, gaseous nitrogen (N2) to ammonium (NH4+) with the help of an estimated 16 ATP molecules and 8 electrons via the rhizobial enzyme nitrogenase (**citation?).** In exchange for “fixed” nitrogen the rhizobia receive a steady stream of carbon (energy) sources making this symbiosis mutually beneficial to both bacteria and host. This phenomenon is found exclusively in legumes (**yes? No?**) and acts as the main method for getting molecularly useful nitrogen into the food chain. Nitrogen is a crucial nutrient that forms the backbone of several important biological molecules including amino and nucleic acids. This ability to supply nitrogen to crops naturally decreases the need for expensive and potentially dangerous fertilizers. Indeed, the more we know about how this process works, the closer we get to being able to engineer nitrogen fixation into non-legumes.

As mentioned above, the process of nitrogen fixation occurs in specialized root organs called nodules. Nodules form after several steps of back and forth communication between the host legume and the invading rhizobia. Briefly, the process begins as roots emit chemical signals in the form of specific flavonoids to attract rhizobia. Upon reception of these signals the bacteria emit their own signals known as Nod factors (nod is short for nodulation) which switch on various processes that prepare the plant for invasion. Nod factors are lipochitooligosaccharides with various modifications at terminal and non-terminal residues. The number of N-acetylglucosamine residues varies, but generally is within the range of 3-5. The host responses to Nod factor recognition involve increases in intracellular Ca2+ in root hairs, followed by Ca2+ oscillations (spiking) and alterations in root hair cytoskeleton leading to root hair curling which traps rhizobia in colonized curled root hairs. At this point, the bacteria produce Nod factors and symbiotically active exopolysaccharides which induce growth of root hair cell membrane towards the cortex and then invasion usually progresses via bacterial proliferation. Nod factors act on host tissues as mitogens and morphogens which reactivate the cell cycle in the host cortex eventually leading to development and differentiation of nodule tissues including vascularization. During this time intracellular, un-walled outgrowths called infection droplets form from infection threads which allow direct contact and subsequent uptake of rhizobia into plant cell cytoplasm. (At some point rhizobia terminally differentiate into nitrogen-fixing bacteroids…) The result is called a symbiosome and its peribacteroid membrane regulates the metabolic exchanges between bacteroids and host cytoplasm.

* 1. Legumes, symbiosis, and *Medicago truncatula*
  2. Plant cell walls
     1. Cell wall polysaccharides
     2. Cell wall proteins
  3. Proline Rich Protein (PRP) family
  4. Functional analysis of the PRPs
     1. Overexpression
     2. RNAi knockdown
  5. PRP4 promoter: cloning and expression results (so far)

1. **Chapter 2**: Genomic organization of the PRP family
   1. *M. truncatula*
      1. >50kb BamHI fragment contains most PRPs
      2. Chromosome 4
      3. Corroborating, supplementing, and annotating newly available sequence data
   2. Compared to other legumes (where available)
2. **Chapter 3**: Expression patterns of PRP1-3 (and PRP4) in transgenic hairy roots
   1. Cloning and subcloning the promoters
      1. From a commercially available BAC library
      2. Using newly available sequence data
   2. Results from GUS fusions in stably transformed *Arabidopsis* seedlings and hairy roots (where available)
   3. Results from GUS fusions in *M. truncatula* hairy roots
      1. Whole roots
      2. Cross sections
3. **Chapter 4**: Introduction: *Arabidopsis thaliana* project
   1. Plant stress response
   2. Desiccation tolerance and ABA
   3. Core ABA signaling pathway
   4. ABI5-binding proteins (AFPs)
   5. The search for AFP-interacting proteins using a yeast two-hybrid screen
      1. Core signaling pathway components
      2. CRK8
   6. Role of AFPs in transcriptional repression via chromatin remodeling
   7. The potential role(s) of AFPs in the core signaling pathway (preliminary data)
4. **Chapter 5**: AFP domain mapping: -A, -AB, -B, -BC, -C
   1. ABA insensitivity of 35S-YFP-AFP lines at germination in stably transformedseedlings
      1. Determining expression levels by Western
   2. Subcellular localization of YFP-AFP proteins
      1. Stably transformed seedlings
      2. Transient overexpression in *Nicotiana benthamiana* leaves
   3. Biomolecular fluorescence complementation versus PP2C/SnRK (and CRK8, HDAC, SAP18?)
5. **Chapter 6**: Conclusions and Future Directions
   1. *M. truncatula* project
   2. *A. thaliana* project