

Potential 290 Research Projects in the Zielinski Lab

Overall focus and approach of the lab: I am interested in understanding how Ca^{2+} -mediated signaling is regulated and integrated with various physiological responses in plants, including: guard cell function, polarized growth (seen in root hairs and pollen tubes), and responses to pathogens and insects. Changes in Ca^{2+} concentration are generated in the cytoplasm and nuclei of all eukaryotes as a second messenger in response to an array of hormonal and environmental stimuli. Our main focus is on understanding how the Ca^{2+} receptor protein calmodulin (CaM) communicates Ca^{2+} signals to help de-code Ca^{2+} signals and elicit physiological responses to stimuli. CaM functions by binding other proteins and modulating their activities. Current estimates are that there are more than two-dozen proteins that specifically interact with CaM; this means that CaM represents a crucial link in the pathways by which Ca^{2+} signals are de-coded.

Opportunities and training for students: Projects in the lab are carried out from the whole plant to the molecular level; individual research projects are tailored to suit the background experience and interests of individual students at all stages of their undergraduate training from beginning to advanced levels. Students will work directly with both graduate students and the principle investigator to be trained in methods needed to carry out the research and in conducting the actual project. Students will participate in informal laboratory meetings where methods and results of work are presented and discussed to gain familiarity and experience in scientific communication; they are also encouraged to present their work in local and regional scientific meetings.

Project: Assessing the effects of CaM knockout mutations in Arabidopsis.

Background: We have identified lines of mutant plants in which different genes encoding CaM have been knocked out by insertional mutation. The goal of this project is to determine whether one particular CaM gene plays a dominant role in controlling several well-characterized Ca^{2+} -mediated responses in plants: guard cell closure, root hair growth and pollen tube growth.

What the project entails: Mutant lines of plants will be maintained and used to: (1) prepare epidermal peels that can be used in stomatal closure assays (recording stomatal aperture over time by digital photography), (2) record root hair growth, and/or (3) recover pollen that will be germinated in vitro to measure its growth.

Project: Constructing transgenic plants that report the expression of genes involved in plant defensive responses to pathogens and insects.

Background: In collaboration with Evan Delucia (Plant Biology) and Art Zangerl (Entomology) we are asking whether the "cost" of turning on defensive genes in plant in response to insect herbivory involves turning off genes involved in producing proteins needed to maintain chloroplasts. My lab's contribution to this project is to introduce gene fusions into transgenic plants that express GFP under the control of "defensive" gene and

"photosynthetic" gene promoters so the spatial pattern of gene expression can be measured in response to insect damage to leaves.

What the project entails: Transforming *Arabidopsis* plants using *Agrobacterium tumefaciens* harboring plasmids that contain promoter-GFP gene fusions, and constructing new gene fusions that can be used to transform plants. Recovering and growing transgenic plants for use in imaging experiments. Assisting in rearing insects and conducting herbivory experiments.

Project: Constructing and maintaining transgenic lines of plants that express fluorescent reporters of CaM function.

Background: Ca²⁺ signaling mediated by CaM is carried out by physical interaction between CaM and other proteins. We have devised a method to measure this interaction using fluorescent fusion proteins that report CaM binding; we call these fluorescent indicator proteins (FIPs). By expressing FIPs in transgenic plants, we are asking whether there are spatial differences in the pattern of CaM activation in cells stimulated to generate Ca²⁺ signals.

What the project entails: Transforming *Arabidopsis* plants using *Agrobacterium tumefaciens* harboring plasmids that contain fluorescent indicator protein gene constructs, and constructing new gene fusions that can be used to transform plants. Recovering and growing transgenic plants for use in imaging experiments by fluorescence microscopic screening of potential transformants.

Project: Improving the sensitivity of green fluorescent reporter genes.

Background: A number of studies have identified mutations in GFP that alter its fluorescence. Many of these are used as reporter genes in a variety of applications in plant and animal systems. Few of these have been systematically compared for the combination of their efficiency of expression and fluorescence, particularly in plants. We would like to identify the best combination of GFP mutations that optimizes GFP's sensitivity as a reporter gene in plants.

What the project entails: Constructing specific site-directed mutations in a GFP reporter gene in use in the lab, expressing these mutants in bacteria, purifying the proteins and measuring their fluorescence properties. Constructing plant expression vectors designed to express the same mutant proteins in plants transiently transformed by microprojectile bombardment.