Undergraduate Research Symposium May 19, 2017 Mary Gates Hall

Online Proceedings

POSTER SESSION 3

MGH 206, Easel 175

2:30 PM to 4:00 PM

When Plants Strike Back: Engineering Plants with Pest-Triggered Defenses Using Synthetic Transcription Factors

Andrew Charles (Andrew) Lemmex, Senior, Biology (Molecular, Cellular & Developmental)
Mary Gates Scholar
Mentor: Jennifer Nemhauser, Biology
Mentor: Arjun Khakhar, Bioengineering

Crop production will have to double by 2050 to meet projected global food demands. Insect herbivory currently accounts for nearly 15% of global crop losses; thus, plants better able to repel insect attack should have a significant impact on food security. One successful strategy for engineering plants is the constitutive expression of insecticidal toxins such as Bacillus thuringiensis endotoxin (BT). However, the constant production of these toxins decreases crop yield and increases the development of BT-resistant pests. When plants are damaged by insects, they produce the phytohormone jasmonate. We propose a new plant engineering strategy that links the expression of BT to jasmonate signaling. To create this link we have built a synthetic transcription factor called a Jasmonate Degradable CRISPR Transcription Factor (JDCTF). This transcription factor is easily retargeted to any gene of interest by changing the dcas9-associated guide RNA. The JDCTF responds to jasmonate via a jasmonate sensitive degradation domain. Using the plant model *Arabidopsis thaliana*, we've generated transgenic plant lines expressing a JDCTF that targets a Venus-Luciferase reporter. We have shown the system works using a bioluminescence time course assay on seedlings treated with the jasmonate analog coronatine. After induction with coronatine, we observed an increase in bioluminescence in the treated seedlings and no increase in biolumescence for the control. Currently we are characterizing the dynamic response to both exogenous jasmonate, as well as simulated and real insect herbivory on mature leaves. Future work will focus on regulating BT expression with the JDCTF system. Our system has the potential to increase yields, increase consumer safety, and slow down the development of insecticide resistance in pests.

POSTER SESSION 3

MGH 206, Easel 176

2:30 PM to 4:00 PM

Controlling the Flow: Re-Engineering the Flow of a Plant Growth Hormone to Tune Developmental Processes

Mrunmayee Manohar Shete, Senior, Biology (Molecular, Cellular & Developmental)
Mary Gates Scholar, UW Honors Program
Mentor: Jennifer Nemhauser, Biology
Mentor: Arjun Khakhar, Bioengineering

Climate change is threatening global food security. There is a pressing need for better crops that can prevent severe losses. We hope to apply our current understanding of plant developmental mechanisms that are involved in responses to harsh external stimuli to engineer more resistant crop varieties. The plant hormone auxin plays an important role in organogenesis, the process of new organ formation. Auxin controls organogenesis by accumulating in specific tissues at specific developmental times. These accumulations are due to the polar flow of auxin through the plant. These flows are in turn reinforced by auxin through a feedback mechanism called canalization. PIN1 is a an auxin efflux transporter that is required for polar auxin transport. Loss of PIN1 results in several developmental defects. These phenotypes and other analyses have led to mathematical models of organogenesis. We used these models to predict plant phenotype in response to increased canalization. We have engineered transgenic plants with a library of synthetic promoters with varying auxin sensitivity driving expression of PIN1. We are characterizing the shoot architectures of these plants, specifically the number of branches and the pattern of lateral organ initiation. We are also studying how the increased canalization affects the distribution of auxin in our plant lines using fluorescent reporters. We hope that our results will demonstrate how plant synthetic biology and predictive models can be used to rationally design plant developmental phenotypes.

POSTER SESSION 3

Commons East, Easel 63

2:30 PM to 4:00 PM

Engineering Novel MAP Kinase Interaction Domains in *Saccharomyces cerevisiae*

Anna Kus, Senior, Anthropology: Human Evolutionary Biology, Biology (Molecular, Cellular & Developmental)
Mentor: Georg Seelig, Electrical Engineering and Computer Science & Engineering
Mentor: Arjun Khakhar, Bioengineering

An organism's ability to sense, interpret, and respond to its environment is an essential requirement of life itself. At the cellular level, this process of connecting signals to behaviors is governed by a diverse set of mechanisms such as mitogen activated protein (MAP) kinase cascades, which are broadly conserved across animals, plants and fungi. Alterations to these connections that arise from evolution or disease can have significant phenotypic effects. MAP kinases transmit signals by colocalizing with a target protein and altering its structure through phosphorylation and thereby producing downstream behavioral changes in the cell. Engineering novel phosphorylation triggered interaction domains will allow MAP kinase signaling networks to be rewired to correct pathological dysfunction or to generate novel responses to environmental signals. We intend to use an engineered protein scaffold with a phosphorylatable motif and an occluded binding motif, which was designed by collaborators in the Baker lab, to create a phosphorylation triggered interaction motif in *Saccharomyces cerevisiae*. By rewiring the *S. cerevisiae* mating MAP kinase cascade to phosphorylate this scaffold and incorporating a yeast three hybrid output, we hope to demonstrate the function of this new class of interaction domains *in vivo*. We hypothesize that upon phosphorylation, the scaffold will undergo a conformational change revealing the previously occluded binding motif, allowing another protein to bind and produce downstream effects. Such novel interaction domains will serve as a "toolbox" to allow the rewiring of MAP kinase cascades in any context in which they naturally occur. This approach could be used to fix pathological miswiring of MAP kinase cascades that contribute to anomalies of development or diseases such as cancer. Additionally, the creation of new connections using these interaction domains could be utilized to engineer organisms or cells with useful functions such as plants with enhanced defensive traits or cancer targeting T-cells.