Cell and Molecular Biology Symposium

Book of Abstracts

Winter 2021

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Polarity kinase Orb6 and its interaction with cytokinesis kinase Cdr2 in fission yeast

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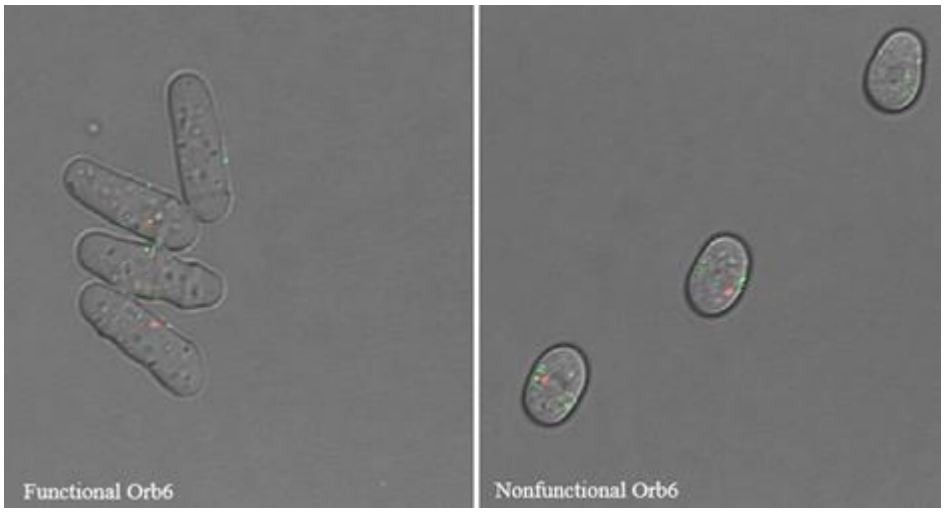

Loss of cell polarity and an altered morphology are hallmarks of cancer. Fission yeast, *Schizosaccharomyces pombe,* undergo polarized growth by extending at the tips and therefore, it is an ideal model organism to study cell polarity and shape. Moreover, *S. pombe* have a similar cellular cycle to human cells, characterized by a distinct polar elongation during interphase followed by a contractile actin-myosin ring formation during division. Many interactions between growth and division cycle proteins have been identified, but the regulations between the two are not fully understood.

Here, we investigate the direct interaction between polarity kinase Orb6 and cytokinesis kinase, Cdr2, to better understand the coordination between the division (Septation Initiation Network) and growth (Morphogenesis Orb6 Network) signaling pathways. To study the regulatory interactions between these two pathways, the *S. pombe* genome was mutated to contain a temperature sensitive *orb6* gene combined with a *cdr2* fluorescent tag. Using confocal microscopy, the localization of Cdr2 was visualized in the absence of functional Orb6. Our current results suggest that when the cells are round due to the inactivation of Orb6, Cdr2 remains associated with the medial cortex and this may interfere with progression of cell division. Orb6 and Cdr2 are signaling proteins from evolutionarily conserved pathways in yeast and human cells. Therefore, identifying this interaction may help to define the molecular signals that promote morphological changes seen in human disease, such as cancer.

Micronuclei Clusters and Genome Chaos: Creating New Genomes Under Crisis

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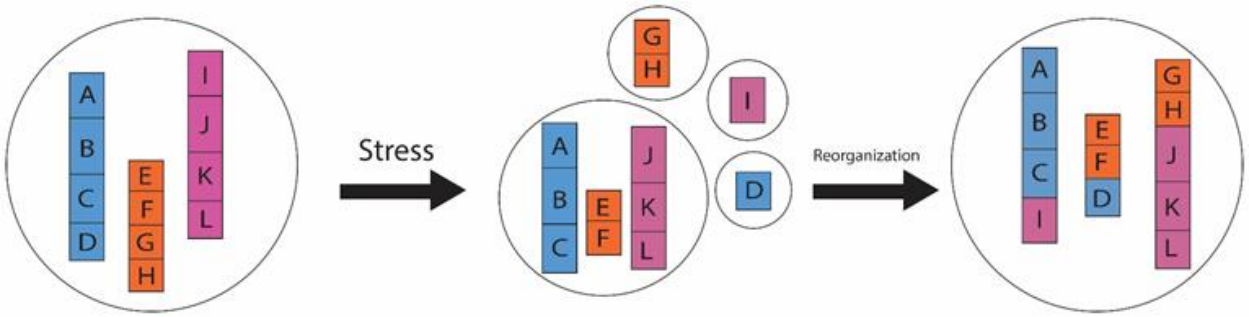
Supported by Summer Undergraduate Research Program, Center for Molecular Medicine and Genomics, Wayne State University School of Medicine.



Micronuclei are small nuclei composed of one or more chromosomes. Micronuclei and micronuclei clusters are often seen as a result of cellular stress, and, along with other nuclear aberrations, can result in genome chaos (massive genome reorganization) which can cause cancer development, evolution, and drug resistance. However, micronuclei have not yet been extensively studied, so the relationship between various types and amounts of cellular stress and stable and unstable cancer cell lines is not clear. In this study, we observed the number of micronuclei, micronuclei clusters, and other abnormal nuclei morphology that occurred in stable and unstable cancer cell lines when exposed to stress from cell overgrowth, bacterial contamination, and doxorubicin treatment. It was found that stable cancer cell lines respond less to cellular stress than unstable cell lines and have fewer abnormal nuclei/micronuclei. We also found that high amounts of cellular stress often result in the death of unstable cancer cell lines. These results help to better understand how micronuclei formation and genome chaos are induced in stable and unstable cancer cell lines.

How the Overexpression of Hal22 Affects Growth and Development in *Candida albicans*

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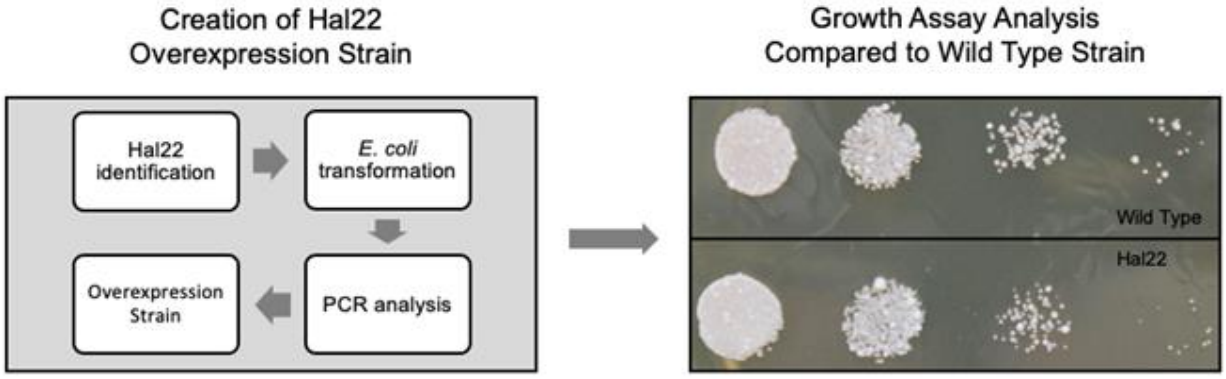

Infections caused by *C. albicans* can become deadly for people with suppressed immune systems. The infections are difficult to diagnose, and it is not fully understood how the filamentous yeast develops. The function of Hal22 has not been determined definitively, but it is known to be induced during biofilm formation. In *C. albicans*, the ability to form a biofilm has been linked to its disease-causing potential and its resistance to antifungal drugs. Here, we investigate the impact of Hal22 on the growth and development of *C. albicans* in different conditions to better understand its function as a gene. A Hal22 overexpressing strain was created and subjected to multiple growth assays, using different hyphae inducing media, and then compared to the SC5314 wild type. The Hal22 overexpression strain showed growth sensitivity when exposed to 3.3% LiCl and 5% NaCl, with higher concentrations inhibiting growth all together. Spider, 20 mM caffeine, SDS, and calcofluor-white, administered individually, also decreased growth in Hal22. In contrast, EDTA, 15 mM caffeine, and 2 M NaCl, administered individually had no impact on the growth of the Hal22 overexpression strain. These results help us to understand the impact of Hal22 on the growth of *C. albicans* in various conditions.

The country and the city lizard: Optimizing microsatellite primers for landscape genetic analyses of a Galápagos lava lizard

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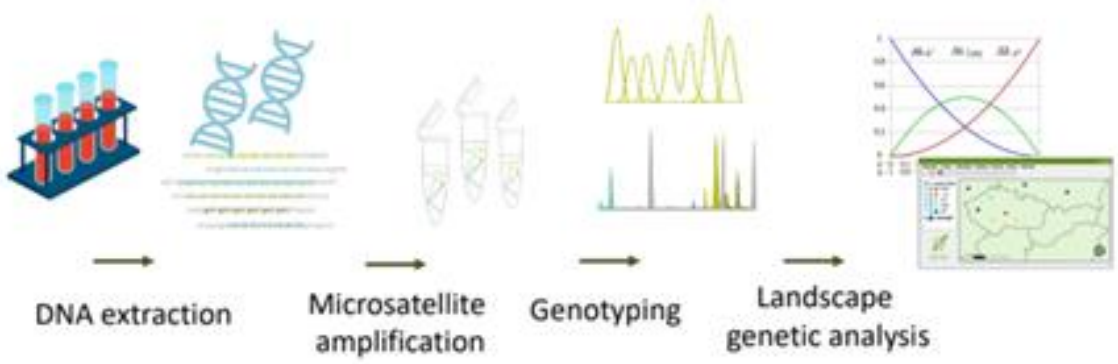

Fauna of the Galápagos Islands is under increasing threat due to the growing demands of human activity. The impact of anthropogenic pressures is unknown for lava lizards (genus *Microlophus*), a group of nine species common throughout the Galápagos. *Microlophus. bivitattus* genetic samples were collected across a gradient of natural and human-modified habitats on the island of San Cristóbal in 2017 and 2018. Our main objective was to understand the ecology and determine the spatial genetic structure of Galápagos lava lizards using microsatellite genotyping. DNA extraction, protocol, and microsatellite primer optimization were carried out. Over 250 DNA samples were extracted, and 8 microsatellite loci were optimized prior to the COVID-19 pandemic. Future work includes PCR amplification of microsatellite loci, genotyping, and landscape genetic analyses to assess relatedness across space and in relation to human modified environments.

This research was supported by the Arnold and Mabel Beckman Foundation and Grand Valley State University.

Molecular modeling of Istradefylline–A_{2A} receptor binding in dopaminergic neurons with implications for Parkinson's Disease treatment

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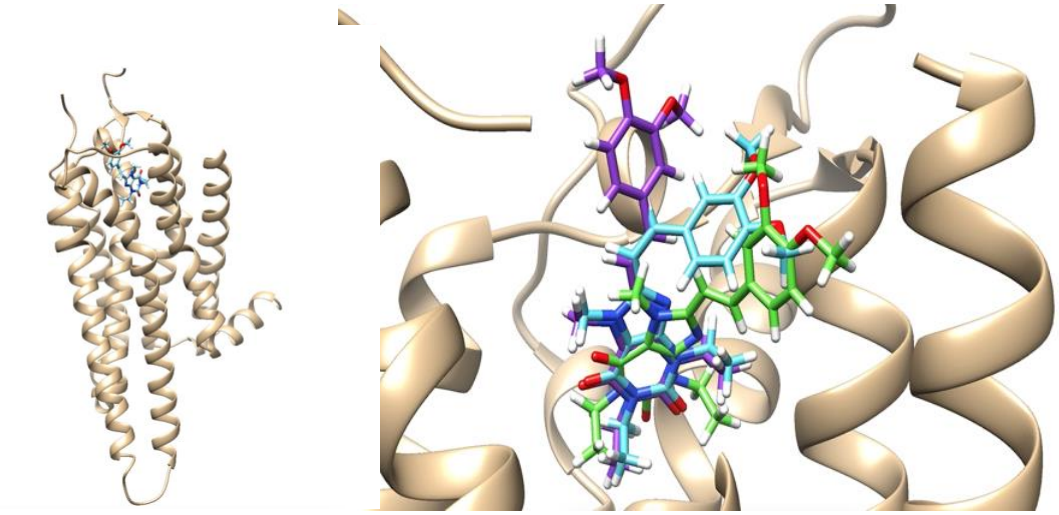

Parkinson's disease (PD) is the second-most common progressive neurodegenerative disease after Alzheimer's disease, with no cure and limited treatments with negative side effects. A new drug, Istradefylline (KW-6002) is a highly selective Adenosine 2A receptor (A_{2A}) antagonist that appears to relieve the disruptive side effects of L-DOPA administration for PD symptom management. However, the mechanism of KW-6002 therapeutic effect remains unclear. In this project, we used homology modeling and protein-ligand docking simulations to determine KW-6002 binding modes to the A_{2A}R. Two experimental structures (PDB IDs 2YDO and 3RFM) and two multi-template homology models used for drug docking with the SwissDock search and scoring algorithm. We found KW-6002 binds to active and inactive receptor structures and is dependent on pi bond stacking with PHE168. Important contacts include LEU167, VAL84, ASN253, MET270, MET177, and GLU169.

The Determination of Phosphorylation of Nato3 by PKA Through Newly Generated Flag Epitopes

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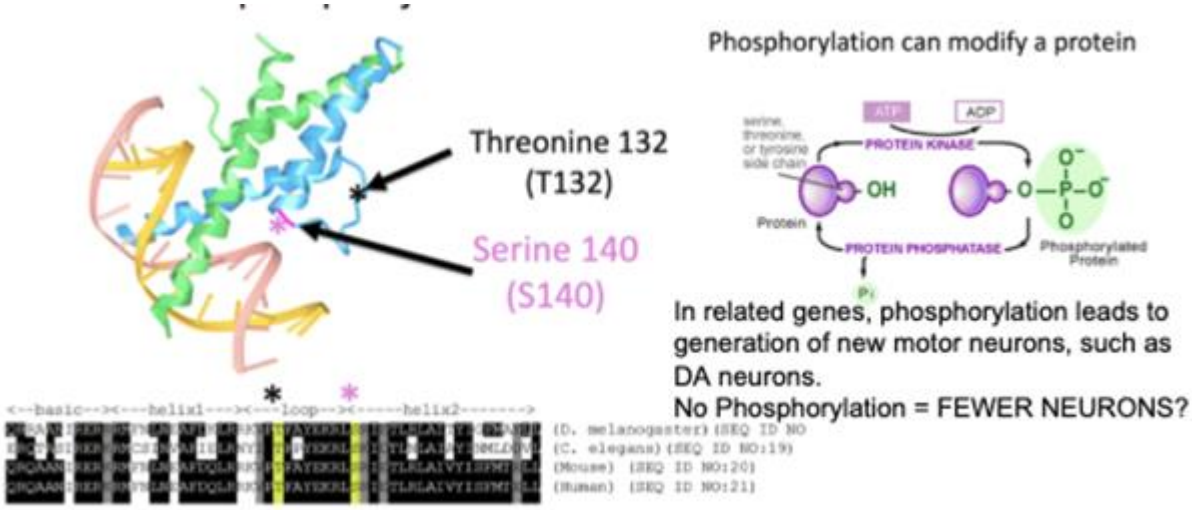

One of the leading regulators of neuronal cell differentiation in the central nervous system (CNS) is the family of basic helix-loop-helix (bHLH) transcription factors. One of these proteins, Nato3, is associated with the formation of dopaminergic neurons. Transcription factors can be regulated through kinase activity and phosphomimetics of Nato3 was shown to induce expression of genes related to dopamine neurogenesis and protection. In this study, in order to detect the associated change of phosphorylation of Nato3, I have generated Nato3 with a specific epitope tag that allows for the detection and isolation of the Nato3 protein. Subcloning techniques and transformation of the Nato3 gene with the sequence of the epitope for the 3X flag or the C-cmyc flag into a pCDNA3.0 vector were used. These epitopes attached to Nato3 will allow us to purify the protein for further analysis by immunoprecipitation, as well as allow for better detection of phosphorylation status in SDS-PAGE mobility assay.

Microbial Exposure Shapes the Release of TNF-α by Macrophages Stimulated with Flagella or Lipopolysaccharide

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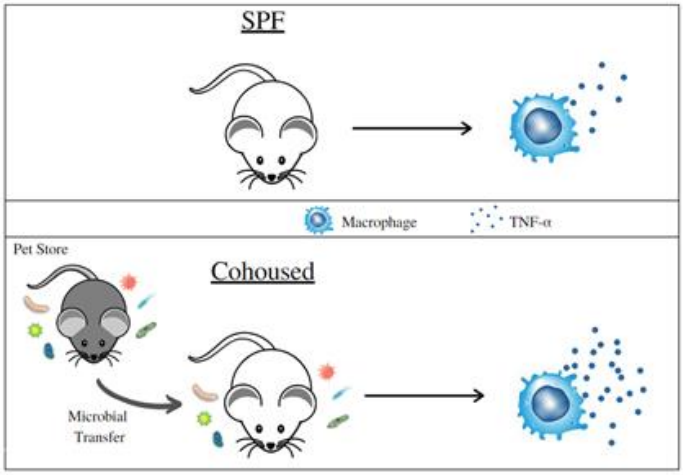

The prevalence of allergies and allergic asthma has rapidly increased in recent decades. Increased hygienic practices may contribute to immune system overreaction to allergens by preventing critical signals from shaping the basal immune state. Cohousing specific pathogen free (SPF) mice with pet store mice, which carry numerous microbes, facilitates natural pathogen exchange, resulting in global changes to the immune system. However, how microbial exposure shapes immune cell signaling pathways throughout development is not clear. Here, we investigate the effects of microbial exposure on the release of tumor necrosis factor alpha (TNF-α) by macrophages stimulated with bacteria-derived flagella or lipopolysaccharide (LPS). In order to study the impact of microbial exposure on inflammatory cytokine release, enzyme-linked immunosorbent assays (ELISA) were conducted on the culture supernatant of stimulated cells from intraperitoneal lavage. These samples were collected from SPF mice and cohoused mice with varying lengths of exposure. The preliminary data revealed the influence of microbial exposure on TNF-α release by macrophages stimulated with flagella or LPS. The initial results suggested a potential trend of a greater concentration of TNF-α in the IP-lavage supernatant of cohoused mice than SPF mice. However, additional experiments are still needed to confirm this relationship. These preliminary results help us to understand the role of microbial exposure in a pro-inflammatory immune response.

Identification of a Novel Homozygous *MAPT* Variant in an Isolated Case of Frontotemporal Dementia

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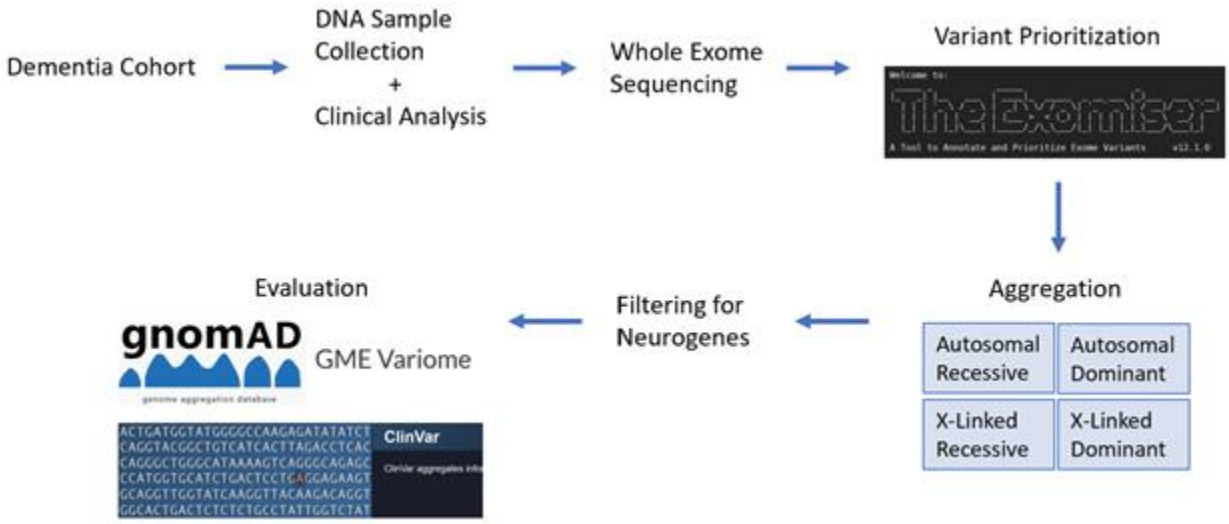

Neurodegenerative diseases are a group of devastating and often fatal disorders with a wide range of etiologies, symptoms, treatments, and life-expectancies. Often, these diseases cause mild cognitive decline which can eventually become dementia. Alzheimer's disease (AD) is the most commonly studied cause of dementia due to its debilitating effects and prevalence, but many other causes of dementia are equally debilitating and less understood. One cause of dementia that is particularly devastating is frontotemporal dementia (FTD), which is characterized by neurodegeneration in the frontal and temporal lobes, causing prominent changes in behavior and personality, loss of language skills, and disturbances in motor function. While strides have been made to understand how and why dementias develop, our knowledge of the etiology of most dementias is lacking. To better understand the genetic causes of dementia, we examined a cohort of approximately 200 Turkish individuals with various neurodegenerative diseases, including AD and FTD. We screened for variants in over 100 genes that had been previously linked to a neurodegenerative disease, and these variants were evaluated for pathogenicity using databases, risk scores, and published literature. Through this process, we discovered a novel, possibly pathogenic *MAPT* variant (Pro605Leu) in a cohort member with FTD. This finding strengthens our understanding of the role that *MAPT* plays in the etiology of FTD and provides a new variant to examine in dementia cases with FTD features.

Analysis of the Effects of Drug Binding in the GABA(A) Ligand Gated Ion Channel

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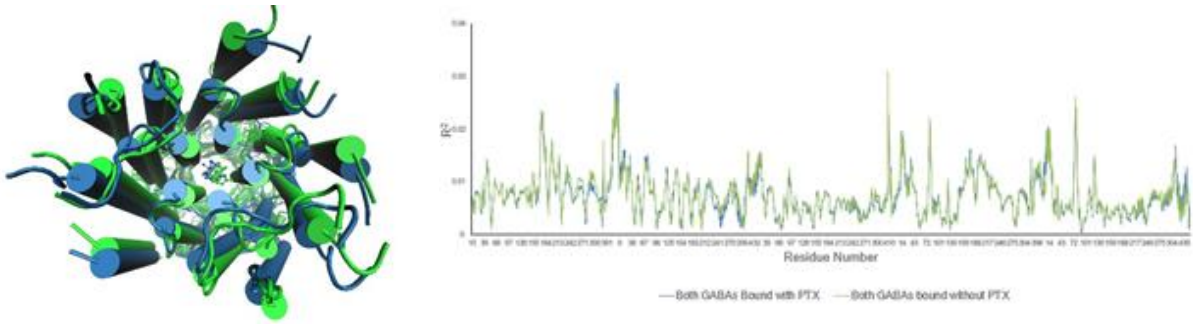

Figure 1A: Mode 30 of GABA(A) receptor bound with picrotoxin (PDB ID: 6HUJ). The green structure represents the first frame, and the blue structure – the final frame of the mode 30 motion. **Figure 1B:** Mode shape 30. Average fluctuations of Cα atoms are shown.

Cys-loop receptors, e.g. GABA(A), mediate fast neurotransmission and belong to a large family of diverse proteins involved in neurological functions. Cys-loop receptors are heteropentamers that assemble in various stoichiometries. GABA(A) receptors are important drug targets for epilepsy, anxiety, and psychosis. They are also involved in general anesthesia. Recently, cryo-EM structures of a human a1-b3-g2 GABA(A) receptor bound with several drugs revealed binding pockets for picrotoxin (non-competitive inhibitor), two different benzodiazepines (positive allosteric modulators), and GABA molecules. However, the mechanisms of action of these drugs and their different effects on GABA(A)R function and dynamics are poorly understood. Here, we used Normal Mode Analysis and structural analysis to identify the vibrational modes of the receptor that are affected by picrotoxin, a channel blocker – and possibly an allosteric modulator, as well as by GABA, the native neurotransmitter. We have found that GABA and picrotoxin do not affect the lowest frequency mode: the global twisting mode suggested previously as the channel gating mode. In contrast, we have identified higher frequency modes, (10, 11, 18 and 30), that are affected by picrotoxin when one or both GABA molecules are bound. These data suggest that higher frequency modes are important for receptor dynamics when channel-bound drugs are present and that, indeed, picrotoxin may have an allosteric effect. We additionally identified key binding residues for GABA and modulator molecules. Taken together, these results will help us understand the mechanisms of receptor's response to various types of effectors.

In Silico Investigation of GABA(A)R-Benzodiazepine Binding Modes

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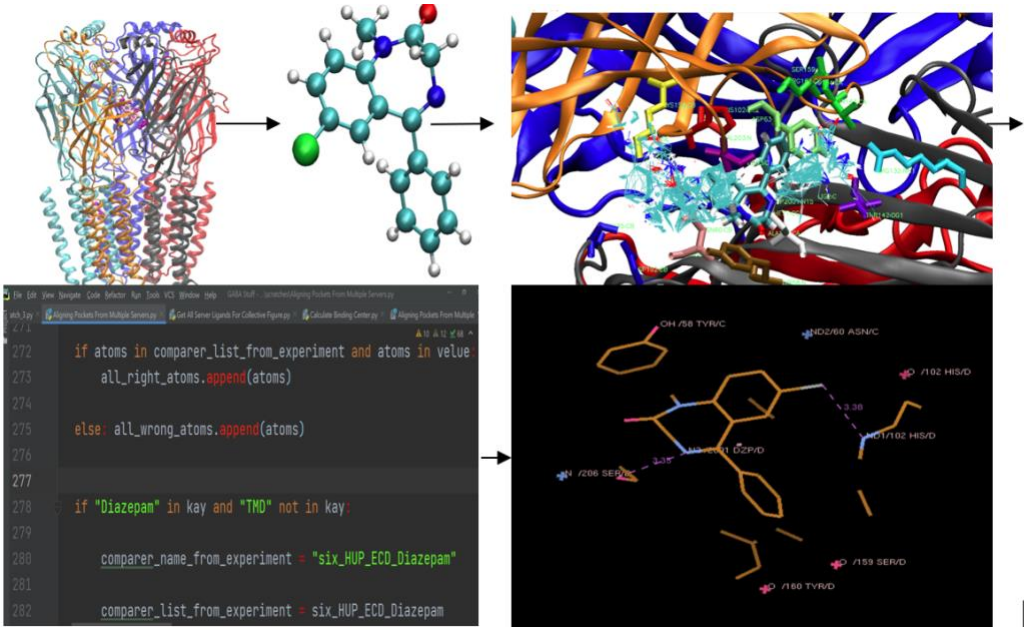

GABA(A) receptors are neurotransmitter-gated chloride channels and pharmacological targets for many drugs, most notably benzodiazepines (BZDs), a class of drugs commonly prescribed as anxiolytics. However, the mechanism of BZD's action is not fully understood. Structures of complexes of these drugs with the human GABA(A)R are available only for diazepam and alprazolam. Knowledge of binding modes of other BZDs would be invaluable for drug optimization and design. To this end, the available structures of GABA(A)R in complex with two BZDs (PDBID 6HUP/6HUO) can be used to benchmark docking protocols for novel drug molecules. As the first step of this study, several search and docking algorithms were tested in order to determine which of these algorithms correctly predicts the binding modes of GABA, diazepam and alprazolam. We tested four different algorithms implemented in web servers: Achilles Blind Docking, PatchDock, Dockthor, and Mcule. We found that interface-targeting yields >50% of complexes in agreement with experimental data while in the all-target searches only 1% of predicted complexes are correct. We concluded that interface-targeting simulations are more suitable to study binding of novel drugs to the BZD canonical pocket, but further research is needed for studies of novel pockets. Next, we carried out docking simulations of ten BZD drugs, five BZDs similar to alprazolam and five BZDs similar to diazepam. We found that several unique binding modes exist, however binding modes involving TYR58 interacting with halogens on the BZD's R7 position remain consistent. Insights gained from this study will help in predicting binding modes of novel BZDs.

Impact of arbuscular mycorrhizal fungal inoculum from till and reduced tilled fields on corn growth and soil health

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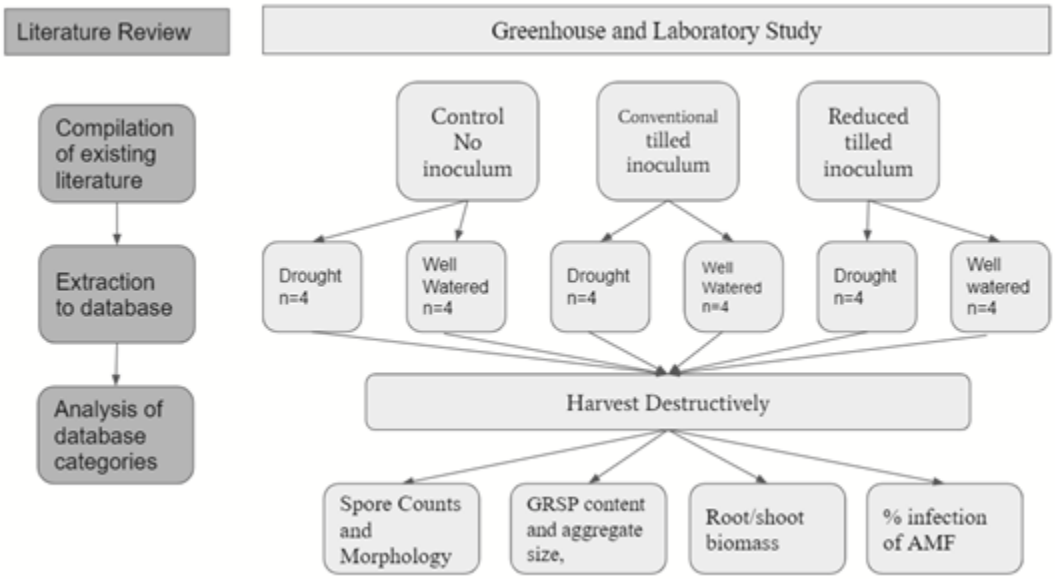

Arbuscular mycorrhizal fungi (AMF) can impact plant productivity and soil health. AMF have a direct link to both plants and soil via their internal and external hyphal networks. The mechanical break up of soil (tilling) is a major agricultural practice that adversely impacts AMF. We set out to better understand the factors that affect AMF in west Michigan, but Covid-19 restrictions during the 2020 growing season meant a need for remote work. A literature review was done, compiling existing work into a database to understand gaps in knowledge. 24 corn plants were grown remotely and inoculated with local AMF from till or no-till fields. After 60 days of growth, the plants and soil were stored until data on growth, spore concentration, AMF infection, and glomalin were able to be analyzed. The experiment taught us about the scientific process while the literature review highlighted how little is known. Experimental results are complicated but suggest that the two types of AMF had mixed interaction with the plants under the different environmental conditions.

Disentangling the role of PntAB, a pyridine nucleotide transhydrogenase, in the regulation of photosynthetic function of flavodiiron proteins in *Synechococcus elongatus* PCC 7942

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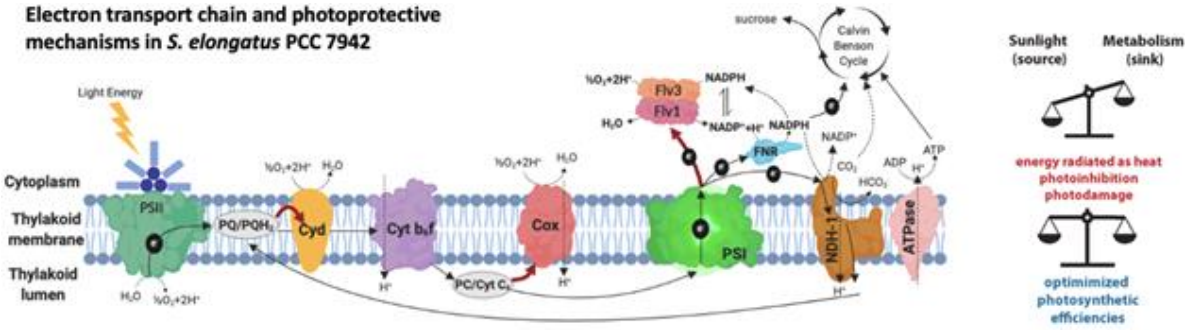

Maximizing the photosynthetic efficiency of cyanobacteria is an important objective that would improve their potential utility for a variety of biotechnological applications. While photoprotective processes help cyanobacteria dissipate excess absorbed light energy, these mechanisms also inherently reduce photosynthetic efficiency. Flavodiiron proteins are an important photoprotective mechanism found in cyanobacteria to safeguard photosystems against oxidative damage, since they deliver excess high energy electrons generated by photosynthesis to O_2 without the simultaneous formation of reactive oxygen species. Our cyanobacterial model species, *Synechococcus elongatus* PCC 7942, has two flavodiiron proteins, Flv3 and Flv1, that have been shown to protect cells under fluctuating light conditions by acting as an electron sink downstream of Photosystem I. The regulation of Flv3/1 is currently unknown but is hypothesized to be influenced by the levels of NADH and/or NADPH in the cell, since both electron carriers can be used as Flv3/1 substrates. We hypothesized that a balance of the ratio of NADH:NADPH is critical to control the activity of Flv3/1, allowing the dissipation of excess energy under stress conditions, but reducing the loss of energy under steady-state light conditions. Pyridine nucleotide transhydrogenase (PntAB) is a key factor involved in balancing NADH:NADPH levels, as it catalyzes the reversible transformation of NADPH into NADH. In this work we explored the hypothesis that PntAB may be involved in regulating Flv3/1, and constructed a mutant lacking PntAB and/or Flv3/1 proteins. We evaluated differences in photosynthetic performance under different light intensities using a custom-built fluorimeter-spectrophotometer. Our results showed that PntAB does not regulate Flv3/1 but plays a role when transitioning from dark to low light conditions. A more detailed understanding of the regulation of Flv3/1 activity may ultimately be useful for reducing the proportion of captured solar energy that is lost to photoprotective mechanisms.

This work was supported by the NSF REU Site award to Plant Genomics @ Michigan State University (award DBI-1757043) and by the Department of Energy (Grant DE-FG02-91ER20021).

Gene-level differential expression analysis of a *Physcomitrella patens* tip growth mutant using OmicsBox

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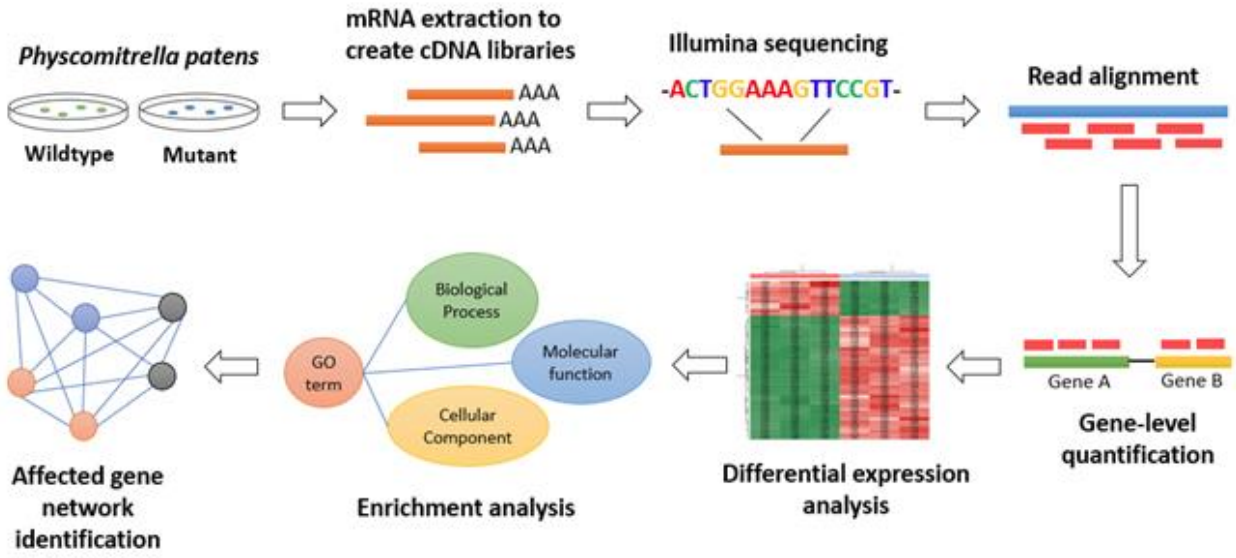

Tip growth is a form of polar growth in which cells elongate in one direction. It plays important roles in plant biology as it is responsible for the formation of root hairs, crucial for nutrient and water uptake, as well as for the elongation of pollen tubes, which enables pollination and fertilization. Previously, we created a random insertional *Physcomitrella patens* mutant whose disrupted locus consists of retrotransposon sequences. No coding sequence seems to be altered, but the mutant phenotype is different from the wildtype in terms of initial cell formation and tip growth patterns. This suggests the mutation has interfered with regulatory regions, although its mechanism is unknown. In this study, we have used OmicsBox, a bioinformatics platform, to determine which genes are expressed differentially to cause the observed phenotypic change in the *P. patens* mutant. The OmicsBox workflow includes genome-guided alignment of the RNA-seq reads, gene-level quantification, and pairwise differential expression analysis. Among 19,784 genes analyzed for differential expression, 967 genes were up-regulated and 1162 genes were down-regulated. Our next step is to perform enrichment analysis on the differentially expressed genes to determine the Gene Ontology annotations, namely cellular components, molecular functions, and biological processes of the encoded proteins. This research will give us insight into the effects of the mutation on gene regulatory network in the moss, the mechanisms of tip growth in plants, and ultimately ways to improve farming practices.

Investigating the Degradation of EIN2 Carboxy Terminal Domain Upon Ethylene Exposure in *Arabidopsis thaliana*

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Mentor: Dr. Matthew Christians[1]

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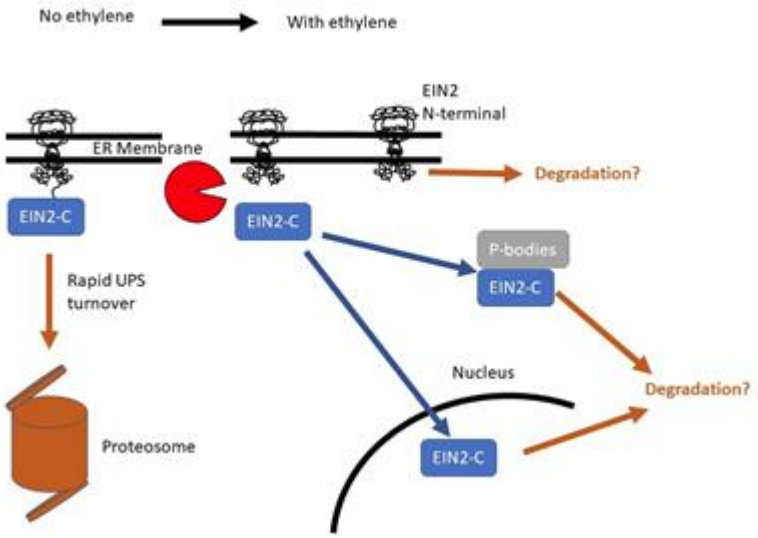

Ethylene is an essential plant stress hormone that regulates development, environmental responses, and immunity and EIN2 is a central protein in this pathway. Soil salinity, resistant pathogens and drought are among many conditions facing farmers and better understanding of plant physiology can lead to higher crop yields in adverse conditions. In low/no ethylene conditions the full-length ER-localized EIN2 protein is rapidly degraded by the proteasome. Upon ethylene exposure, the full-length protein is stabilized and cleaved into two distinct domains by an unknown protease. The amino terminal domain remains in the ER while the carboxy terminal domain (EIN2-C) translocates to the nucleus as well as complexes with P-bodies. The exact mechanism responsible for resetting the ethylene signal is currently unknown. In this study, we utilized SDS-PAGE of etiolated *Arabidopsis thaliana* seedling tissue to better understand EIN2-C turnover. Preliminary results support the stabilization of full-length EIN2 upon ethylene exposure. However, the accumulation or degradation of EIN2-C has been difficult to measure with Western Blot analysis. Further optimization of the protocol is necessary to better determine full seedling levels of EIN2-C as well as the membrane-bound N-terminal domain. In conjunction with full length EIN2 protein accumulation, a novel discovery in the lab indicates that the proteosome subunit, PBA1, is also stabilized upon ethylene exposure. Crosstalk between ethylene and other phytohormones and how these affect caspase-like activity is poorly understood. These results are an important step closer toward understanding the downstream targets of ethylene and how ethylene signaling resets in plant cells.

The Potential Role of the COP9 Signalosome within the Ethylene Signal Transduction Pathway

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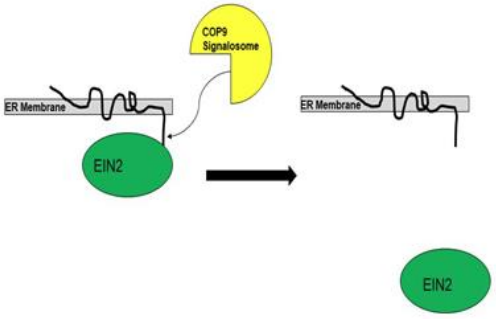
Ethylene is a hormone involved in development, senescence and signaling between plants. Often referred to as the plant ripening hormone or the stress response signal, ethylene has been extensively studied yet its full mechanism of action is not fully understood. One of the first steps of the ethylene signal involves the cleavage of EIN2 (Ethylene Insensitive Protein 2) being cleaved from the endoplasmic reticulum. The cleaved C-terminus end of the EIN2 protein acts as a positive regulator of the ethylene signaling cascade by interacting within the nucleus and with P-bodies within the cell, while the N-terminus end is retained in the ER. The exact mechanism of cleavage is unknown, but we postulate that a multi subunit protein known as the COP9 Signalosome catalyzes it. In this study, potential inhibitors of the COP9 were tested on agar plates using the *Arabidopsis thaliana* as our model organism. Ethylene responses are analyzed using phenotypic changes known as the triple response. These changes include short hypocotyls, fat hypocotyls, and long roots. Our initial data showed the addition of COP9 inhibitors could recover an ethylene response to look like normal growth. These observations suggest the COP9 Signalosome acts in the regulation of EIN2 cleavage and further, ethylene response. If these preliminary results are confirmed, we will have identified an important catalytic mechanism in the ethylene response pathway, extending the current understanding of the hormone.



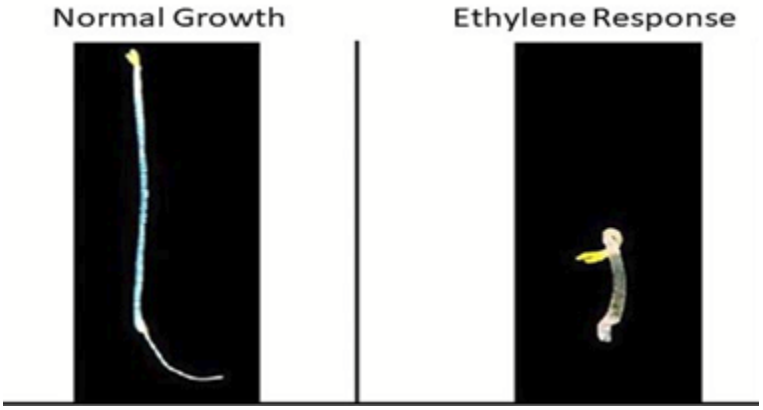


Image Reference
Chen, Yi-Feng, et al. “Ethylene Signal Transduction.” *OUP Academic*, Oxford University Press, 7 Mar. 2005, academic.oup.com/aob/article/95/6/901/188924.

Late Embryogenesis Abundant Proteins in *Arabidopsis Thaliana*

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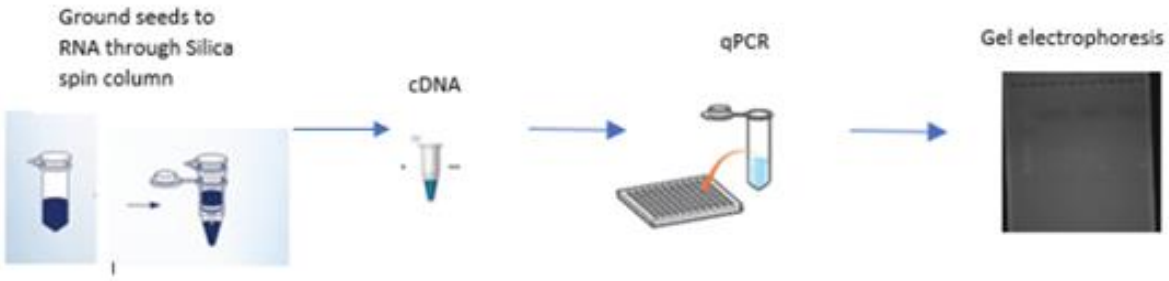
Dr. Sheila Blackman, Joann Thompson

Grand Valley State University, CMB Department



The world relies on seeds stored in long-term seed banks for crop production. The best way to ensure seeds remain germinable is to store them in the desiccated state (less than 0.1 g water/g dry mass). The ability to survive such extreme desiccation is a feature of many flowering plants. We are interested in the factors that allow seeds to germinate after such extreme desiccation. To this end, the expression of the gene for one of the groups of hydrophilic proteins known as Late Embryogenic Abundant (LEA) proteins were studied as they are believed to play a crucial role in germination after desiccation. Seeds (especially minute seeds as are produced by our study organism-*Arabidopsis Thaliana*) are an especially difficult study material for gene expression as storage proteins and oils interfere with common RNA extraction protocols. My work focused on the development of RNA extraction protocol that allows efficient recovery of high-quality RNA suitable for downstream analysis by qPCR. I developed a protocol involving liquid nitrogen freezing followed by a silica spin column. I found that the extracted RNA was suitable for testing via qPCR for LEA protein levels. This protocol will be used to test the link between gene expression and germination rates following germination.

Sequence and motif analysis of SLY1 and other F-box proteins

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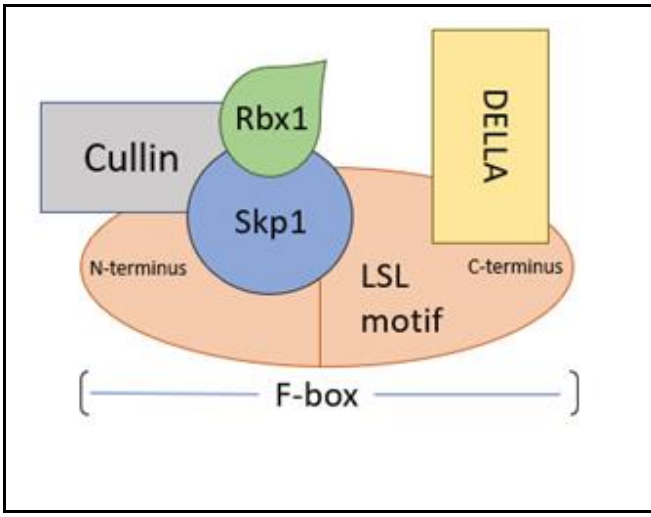

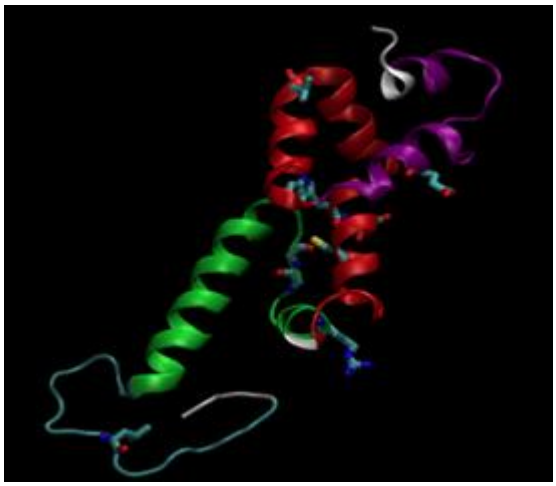

Growth and development of plants depends on the hormone gibberellin (GA). GA signaling is negatively regulated by DELLA proteins, which need to be degraded for the GA-dependent genes to be expressed. The protein necessary for the degradation of DELLAs is SLY1, a member of the F-box family. SLY1, participates in a protein complex SCF that carries out ubiquitination, targeting DELLA. The N-terminal segment of SLY1 F-box domain binds SKP1to form SCF, whereas the C-terminal segment binds the DELLA protein. In this project we aimed to identify the conserved sequence motifs in a dataset of F-box proteins from a variety of species, in order to determine the N-terminal and C-terminal motifs within the F box sequence and structure. A dataset of 93 protein sequences was analyzed for similarities in motifs using multiple sequence alignment and MEME suite to calculate conserved motifs. Sequence motifs were mapped onto the homology model of *A. thaliana* GID2 and SNE proteins. We have found that the LSL is part of a conserved motif longer than previously reported in the literature and is conserved throughout many diverse species of plants. This conserved motif is found on a flexible loop structure, instead of on a secondary structure like other conserved motifs in the F-box sequences, indicating the region of DELLA protein binding. The other conserved motifs could be potential binding sites for SKP1 binding. This data will help to further the understanding of the mechanisms of plant growth, and the role of F-box proteins in this process. It is of significance to plant growth, agriculture, and crop yield.