2023 NIH Protein Biotechnology Symposium Poster Information
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Session 1: 11:45 am – 12:30 pm

Poster #1: Conformational Changes in Herpes Simplex Virus Glycoprotein C Associated with Viral Entry via an Endocytosis Pathway

Author: Katrina Gianopulos

Abstract

Herpesviruses mediate entry by a multi-component, virus-encoded machinery. Herpesviral entry proceeds via endosomal low pH and pH-neutral mechanisms in a cell-specific manner. HSV mediates cell entry via envelope glycoproteins gB, gD, and the heterodimer gH/gL regardless of pH or endocytosis requirements. Conformational changes in the core herpesviral fusogen gB are critical for membrane fusion. We recently demonstrated that gC selectively facilitates low pH entry by positively regulating conformational changes in gB (Komala Sari et al. 2020, mSphere 5: e00826-19). These are novel functions for gC, independent of gC's role in attachment to cell surface heparan sulfate. Here we show that mildly acidic pH, such as that present in epithelial cell endosomes, specifically triggers entry-associated conformational changes in gC itself. Virions were exposed to mildly acidic pH in vitro and conformational changes were monitored by immunoblot using a panel of monoclonal antibodies (MAbs) that recognize distinct epitopes across gC. Changes in gC had a pH threshold of ~ 6.5, similar to low pH-triggered changes in gB. Antibodies to gC epitopes that undergo conformational change selectively inhibited HSV entry by an acidic endosomal pathway, and had less of an effect on pH-neutral entry, suggesting that conformational changes in gC correlate with pathway-specific entry. These epitopes were mapped to the gC N-terminus (residues 23-123). Notably, a panel of gB MAbs inhibited HSV entry into cells regardless of the entry pathway supported by the cell. Our results support a model in which changes in the N-terminus of gC activate the fusion complex of HSV to promote penetration from the epithelial cell endosome.

Poster #3: Characterizing the role of TOP1 in transcription-associated mutagenesis (TAM)

Author: Vanessa Lopez

Abstract

Mutations are changes to a DNA sequence, which are the underlying cause of carcinogenesis. Cancer cells have elevated mutagenesis, and this genome instability contributes to metastasis, cancer progression, and resistance to cancer therapeutics. Though there are many forms of DNA damage, DNA-protein crosslinks are especially harmful because of their bulky protein

component. DNA-protein crosslinks are often formed from reaction intermediates of enzymes that act on chromatin. Topoisomerase 1 (TOP1) creates DNA-protein intermediates called TOP1 cleavage complexes (TOP1ccs), which result from TOP1 acting on DNA to remove supercoiling during transcription. In yeast, TOP1 activity has been shown to contribute to transcription-associated mutagenesis (TAM) and a mutation signature of 2-to-5bp deletions at tandem repeats. In humans, when TOP1 cleaves at unresolved embedded ribonucleotides in the genome, it causes the ID4 mutation signature of small deletions. However, additional signatures associated with TOP1 and the contribution of TOP1ccs to mutagenesis in cancer require further investigation. Our central hypothesis is that TOP1-mediated mutations generate mutations that drive carcinogenesis. Our primary aim is to characterize the TOP1-induced TAM independent of defective ribonucleotide excision repair and to determine the contribution of this process to mutagenesis in human cancer. PARP1 plays an essential role in single-strand break repair but has also been shown to aid in removing TOP1ccs. Therefore, we also aim to determine if PARP1 reduces TAM in human cells and if PARP1 inhibitors could potentially increase TOP1-dependent TAM. We will utilize a novel mutation reporter in a U2OS-derived cell line to accomplish these aims.

Poster #5: Hypochlorous acid produced at the counter electrode inhibits catalase and increases bactericidal activity of a hydrogen peroxide generating electrochemical bandage

Author: Md Monzurul Islam Anoy

Abstract

Previously, an electrochemical bandage (e-bandage) that uses a three-electrode system to produce hydrogen peroxide (H_2O_2) electrochemically on its working electrode was developed as a potential strategy for treating biofilms; it showed activity in reducing biofilms in an agar biofilm model. Xanthan gum-based hydrogel, including NaCl, was used as the electrolyte. While H_2O_2 generated at the working electrode in the vicinity of a biofilm is a main mechanism of activity, the role of the counter electrode was not explored. The goal of this research was to characterize electrochemical reactions occurring on the counter electrode of the e-bandage. Counter electrode potential varied between 1.2 and 1.5 $V_{Ag/AgCl}$; ~125 µM hypochlorous acid (HOCl) was generated within 24 h in the e-bandage system. When HOCl was not produced on the counter electrode (achieved by removing NaCl from the hydrogel), reduction of *Acinetobacter baumannii* BAA-1605 biofilm was 1.08 ± 0.38 $\log_{10}$ CFU/cm^2 after 24 h treatment, whereas when HOCl was produced, reduction was 3.87 ± 1.44 $\log_{10}$ CFU/cm^2. HOCl inhibited catalase activity, abrogating H_2O_2 decomposition. In addition to H_2O_2 generation, the previously described H_2O_2-generating e-bandage generates HOCl on the counter electrode, enhancing its biocidal activity.

Poster #7: The differential response to nitrate availability in the root hairless mutant , buzz, is partially due to the misregulation of the nitrate transporter NRT1.1A

Author: Miguel Rosas

Abstract

Recently, a nitrate responsive cyclin-dependent kinase(CDK)-like gene termed BUZZ was identified in Brachypodium distachyon as a key regulator of root hair growth. Although some studies have investigated the role of root hairs on root system architecture in response to nitrate availability, a link between root plasticity in response to nitrate availability and CDK-mediated root hair growth has never been described. To define the connections between root hair development and physiological responses to nitrate, the root hairless mutant buzz was used to quantify root architectural parameters as well nitrate content in both roots and shoots. The results demonstrate that buzz primary roots are insensitive to nitrate while lateral roots are more sensitive to nitrate. Interestingly, under low nitrate conditions buzz mutants had a significantly higher nitrate content in shoot tissue relative to wild type presumably because the nitrate transporter NRT1.1A is upregulated under low nitrate levels. Moreover, BUZZ partially co-localizes with NRT1.1A in root hairs. These data suggest that the differential response to nitrate between buzz and wild-type is in part attributed to the misregulation of nitrate signaling in buzz. Overall, these data lend insight into root developmental plasticity in response to nitrate in grasses.

Poster #9: Topology of 8-oxo-Gunanine induced mutations reveals impacts of chromatin features on BER.

Author: Cameron Cordero

Abstract

Mutation topology describes how chromatin features, repair processes, and preferential lesion formation impacts the location of mutations across the genome and will aid in the identification of mutation hotspots and accurate differentiation of cancer driver mutations from passenger mutations. In this study, we investigated the factors contributing to 8-oxo-Guanine mutagenesis using a mutation accumulation method and whole genome sequencing of human cells, which yielded over half a million mutations. Analysis of this novel dataset found 8-oxo-G mutations displayed no transcriptional asymmetry, moderate replication asymmetry, and significant effects due to chromatin state and nucleosome positioning. Notably, a higher frequency of mutations occurred at the minor-in position within nucleosomes, indicating that chromatin accessibility inhibits BER removal of 8-oxo-G, leading to higher mutation densities. Interestingly, transcription factors, which bind DNA less strongly than nucleosomes, did not impact 8-oxo-G mutagenesis. These results were further supported by 8-oxo-G lesion mapping data, which

indicated a more uniform distribution of lesion formation. This knowledge aids in distinguishing cancer driver mutations, enabling precise identification of therapeutic targets.

Poster #11: Kinetic Modeling of T Cell Growth and Effector Activity in a Novel Centrifugal Bioreactor with Applications in Cancer Immunotherapy

Author: Brenden Fraser-Hevlin

Abstract

Cancer is one of the leading causes of death worldwide. Traditional treatments like chemotherapy and radiation destroy healthy cells in the process of attacking cancers. A newer alternative is immunotherapy, where killer T cells are sampled from a patient and modified to target their cancer, before being reintroduced into their blood. Immunotherapy is extremely promising, but costly. To resolve bottlenecks in the cell expansion process, our group recently developed a centrifugal bioreactor (CBR) which incorporates a unique balance of forces to quickly reach high cell densities. We recently optimized the design of the CBR and have now begun identifying mechanisms of T cell effector activity related to culture conditions in the system. Literature shows that low availability of glucose and oxygen to cells contributes to metabolic changes including exhaustion, where cells lose their killing ability. We hypothesize that regulation of glucose and oxygen levels in the CBR impacts cell phenotype and can be modeled mathematically. Here we report on the results of flow cytometry assays used to evaluate phenotypic changes, and new CBR models based on these results. In summary, the development of the CBR with optimal growth models will provide a groundbreaking advance in the field of immunotherapy.

Poster #13: SARS-CoV-2 and SARS-CoV-2 Spike protein S1 subunit Trigger Proinflammatory Response in Macrophages in the Absence of Productive Infection

Author: Grace Miller

Abstract

One of the hallmarks of critically ill COVID-19 patients infected with SARS-CoV-2 is exaggerated inflammatory response. Though macrophages mediate inflammatory responses and can produce pro-inflammatory cytokines to eliminate pathogens, infection with SARS-CoV-2 has been shown to cause immune dysfunction, leading to hyperinflammation in the lungs. To further understand the role of macrophages in hyperinflammatory responses during SARS-CoV-2 infection, we infected a THP-1 human derived macrophage cell line with SARS-CoV-2. Our results show that, though macrophages do not support viral replication, infection with SARS-CoV-2 still results in the upregulation of the mRNA of cytokines TNFÎ± and CXCL10, which are markers of COVID-related hyperinflammation. In addition, we identified SARS-CoV-2 Spike protein S1

subunit as one viral factor involved in the upregulation of cytokines in macrophages. We show that glycosylated, soluble S1 protein can upregulate TNFÎ± and CXCL10 mRNAs, as well as the secretion of TNFÎ±, in macrophages in the absence of virus infection. Therefore, macrophage activation by the S1 subunit and SARS-CoV-2 infection may contribute to the hyperinflammation in the lungs seen in critically ill patients through the upregulation of proinflammatory cytokines such as TNFÎ± and CXCL10.

Poster #15: Dissecting the unfolded protein response gene regulatory network in Ixodes scapularis during infection

Author: Kaylee Vosbigian

Abstract

In 2019, over 50,000 tickborne disease cases were reported to the CDC. Of these cases, 68% were caused by the Lyme disease spirochete, Borrelia burgdorferi, and 15% due to Anaplasma phagocytophilum, the causative agent of anaplasmosis. Both pathogens are transmitted by the North American deer tick, Ixodes scapularis. Most research on arthropod immunity has been modeled in Drosophila. However, immunity is divergent across arthropod species. How I.scapularis immunity influences vector competency is incompletely understood. The unfolded protein response (UPR), a cellular stress response has recently been linked to tick immunity. This led to our current question: how does the UPR gene regulatory network respond to infection and influence pathogen dynamics in ticks? Using a UPR reporter plasmid assay, we determined that the transcription factors, ATF6 and NRF2, were activated by infection. To predict ATF6 and NRF2-regulated genes, we designed a computational binding site prediction model of the I.scapularis genome. Based on this information, chosen genes were knockdown in tick cells using RNAi during infection with A.phagocytophilum. Our results suggest ATF6 and NRF2-regulated genes effect A.phagocytophilum levels. Overall, we have generated a novel approach for predicting gene regulatory networks and discovered new factors influencing infection in tick cells.

Poster #17: Defining the epigenetic regulation of fibroblast lineages during embryonic development

Author: Quan Phan

Abstract

Dermal Fibroblast Progenitors (DFPs) differentiate into distinct fibroblast lineages during skin development. However, the mechanisms that regulate lineage commitment of naive dermal progenitors to form niches around the hair follicle, dermis, and hypodermis, are unknown. In our study, we used multimodal single-cell approaches, epigenetic assays, and allografting

techniques to define a DFP state and the mechanisms that govern its differentiation potential. Our results indicate that the overall chromatin profile of DFPs is repressed by H3K27me3 and has inaccessible chromatin at lineage specific genes. Surprisingly, the repressed chromatin profile of DFPs renders them unable to reform skin in allograft assays despite their multipotent potential. Distinct fibroblast lineages, such as the dermal papilla and adipocytes contained specific chromatin profiles that were de-repressed during late embryogenesis by the H3K27-me3 demethylase, Kdm6b/Jmjd3. Tissue-specific deletion of Kdm6b/Jmjd3 resulted in ablating the adipocyte compartment and inhibiting mature dermal papilla functions in single-cell-RNA-seq, ChIPseq, and allografting assays. Altogether our studies reveal a mechanistic multimodal understanding of how DFPs differentiate into distinct fibroblast lineages.

Poster #19: Acyltransferases with Desirable Substrate Specificity for Engineered Taxol Biosynthesis

Author: Kaylie Barton

Abstract

Taxol is currently being used to treat medical conditions such as breast cancer, non-small-cell lung cancer, head-and-neck cancer, AIDS-related Kaposi's sarcoma, and most recently is being explored as a treatment to halt the progression of Alzheimer's disease and tuberculosis (4-9). The wide range of clinical applications has created a high demand for Taxol and its analogs, but the compound is still extracted non-sustainably from the bark of Taxus trees. A biotechnological taxol production, such as the expression of all taxol biosynthetic genes in engineered microbes, would be desirable as an alternative, but the corresponding enzymes characterized thus far lack specificity. N-debenzoyl-2'-deoxypaclitaxel: N-benzoyltransferase (NDTBT) is the enzyme responsible for catalyzing a condensation reaction between an aroyl/alkyl-CoA ester and N-debenzoyl-2'-deoxypaclitaxel. Performing kinetic assays with NDTBT and a wide range of alkanoyl/alkenyl substrates will allow us to gain more insight into the specificity of the enzyme. We will also aim to identify the catalytic site residues by performing L-alanine scanning mutagenesis. To further inform these efforts, we will attempt to trap a nonhydrolyzable CoA, propionyl-aza(dethia)coenzyme A, in NDTBT and generate a crystal structure of the enzyme-ligand complex. Residues identified as being likely active site residues will then be subjected to site-saturation mutagenesis to generate enzyme variants with higher specificity for the formation of a specific taxane product. If necessary, multiple mutations will be introduced to enhance NDTBT specificity.

Poster #21: DIRECT PROTEIN INTERACTION MEASUREMENTS OF THE PHENYLPROPANOID PATHWAY IN SORGHUM BICOLOR IN A BIOMIMETIC LIPID BILAYER

Author: Eric Jacobo

Abstract

Phenylalanine ammonia-lyase (PAL) is the first enzyme of the phenylpropanoid pathway followed by cinnamate 4-hydroxylase (C4H), a cytochrome P450 monooxygenase associated with the endoplasmic reticulum of plant cells. PAL and C4H are important for the plant cells due to their involvement in kickstarting the production of primary and secondary metabolites such as phenylpropanoids and lignin. C4H also interacts with cytochrome P450 reductase (CPR; Sorghum bicolor CPR in this study) to hydrolyze trans-cinnamic acid into p-coumaric acid. This study uses fluorescence correlation spectroscopy and single-protein fluorescence tracking of labeled proteins to directly measure the interactions of all three proteins in the absence and presents of their respective substrates and measure protein lipid interactions as a function of state (i.e. oxidation state, heterodimeric state, substrate occupancy, etc.)

Poster #23: Sequential nature of fusion-associated conformational changes in herpes simplex virus envelope glycoprotein B

Author: Albina Makio

Abstract

Herpes simplex virus (HSV) is a ubiquitous human pathogen that causes significant morbidity and mortality. HSV enters host cells by low endosomal pH-dependent or pH-neutral mechanisms in a cell-specific manner. The HSV fusion mechanism is a complex interplay of several virus and host factors. Intracellular low pH in the host cell triggers conformational changes in the core HSV fusogen, glycoprotein B (gB), which drives the fusion process and ultimately virus entry. How and when low pH affects gB conformation is not clear. We hypothesize that endosomal pH triggers early conformational changes in gB that result in the formation of an extended intermediate required for fusion and entry of HSV. In Specific Aim 1, I will elucidate the antigenic reactivity of HSV-1 gB in a pre-fusion conformation. For Specific Aim 2, I will delineate the role of mildly acidic low pH in the formation of the extended intermediate. To accomplish these goals, I will generate point mutations in gB by site-directed mutagenesis and will implement assays to quantitate virus fusion, viral entry, gB antibody reactivity, and gB conformational changes. Completion of the aims will yield a detailed understanding of gB's transition to the post-fusion state, which mediates the membrane fusion reaction and viral entry. The results will enable a detailed understanding of the HSV fusion mechanism and will improve the knowledge base of potential targets for interventions.

Poster #25: Influences of Mitochondria on Dietary-induced Ferroptosis in C. elegans

Author: Jimena Ruiz

Abstract

Ferroptosis is a form of regulated cell death driven by the accumulation of iron-dependent lipid ROS. It has been associated with several neurodegenerative diseases such as Alzheimer's and Parkinson's disease, in addition to cancer, kidney injury, and cardiovascular diseases. Lipid peroxidation is a hallmark of ferroptosis which directly destroys cellular membranes, leading to cell death. The role of mitochondria in ferroptosis is poorly understood and there is much debate as to whether mitochondria are involved in the regulation of ferroptosis. The Watts lab has established that dietary-DGLA leads to ferroptosis in the Caenorhabditis elegans germline and causes sterility. Because mitochondria are an abundant source of ROS generation and there is a plentiful amount located in the worms' germ cells, we suspect it can play a role in lipid ROS accumulation in ferroptosis. We are working with mutant strains of C. elegans with reduced mitochondrial functions and examining their lipid composition to identify any influences mitochondria have on dietary-induced ferroptosis in the context of a whole organism. With this study, we strive to bring more insight into the roles of mitochondrial-generated ROS and mitochondrial lipids on dietary-induced ferroptosis in the worm model.

Poster #27: Studying effects of disease-related mutations in progranulin using molecular dynamics simulations

Author: Eduardo Sanchez Diaz

Abstract

Progranulin is a secreted glycoprotein of 75-80 kDa, containing 7.5 granulin modules (p,G,F,B,A,C,D and E) which are encoded by the GRN gene. Mutations within the GRN gene have been linked to the development of frontotemporal lobar degeneration (FTLD). FTLD is an early-onset dementia syndrome characterized by progressive decline in behavior or language. So far, the effect of the mutations on the structural characteristics and unique cysteine-cysteine pairing pattern of granulin modules is unclear. Using molecular dynamics simulations (MDS), we studied effects of the W541C mutation in granulin module E on stability and disulfide bond patterns. For this, we used the known structure of the zebra fish granulin (pdb # 6cku), a stack of three Î²-hairpins stabilized by a total of six disulfide bonds, which has high homology to granulin E. The mutation was introduced in silico using UCSF Chimera. The 1000ns MDS were run for the wild-type protein and mutated protein, either with all six disulfide bonds intact or with two of them reduced. First, we studied propensity of disulfide bonds in the wild-type protein. Then we compared structure and bond formation for wild-type and the mutant. Our results demonstrated that formation of the disulfide bond between C10 and C26 is disfavored due to the mutation.

Poster #29: Understanding photosynthetic performance in oil-accumulating tobacco leaves

Author: Brandon Johnson

Abstract

A phenomics approach was utilized to understand the effects of oil-engineering vegetative tissues to accumulate up to one third of leaf dry weight as oil. The purpose of this investigation was to uncover differences in photosynthetic performance in three genotypes of oil accumulating lines, namely the High Oil line (HO), SDP1_ RNAi, and LEC2 tobacco, as well as the wild-type (WT) line. The HO background produces 15% oil per leaf dry weight and serves as the parent background for the other two genotypes (SD1_ RNAi, and LEC2) which both produce 30% oil per leaf dry weight, albeit by different metabolic engineering strategies. Interestingly, the HO line has a stunted growth phenotype and suffers from an oil-to-starch futile cycle which limits total oil yields, however, HO has the most efficient linear electron transport and least amount of nonphotochemical quenching of all the genotypes (including WT). This trend does not correlate to the amount of oil nor is there a clear solution to explain this anomaly. Herein, I have illustrated several potential mechanisms that could explain the discrepancies in photosynthetic performance.

Session 2: 1:30 pm – 2:15 pm

Poster #2: Investigation of host-dependent therapeutic drug will uncover previously unknown Francisella tularensis-host interaction

Author: Shannon Allen Whiles

Abstract

Francisella tularensis (FT) is a facultative intracellular bacterium which causes the disease tularemia, also known as rabbit fever. FT is considered one of the most virulent known pathogens, is endemic in North America, and is CDC-classified as a Tier 1 potential bioweapon. Currently, no Tularemia vaccine is available. Upon diagnosis of the disease, antibiotics such as doxycycline, ciprofloxacin, streptomycin, and gentamicin are administered, all of which are associated with toxicity, adverse reactions, disease relapse, and/or resistance. The precise mechanisms which FT employs to evade immune detection and cause disease are poorly understood, with only one officially characterized FT effector protein. This research aims to elucidate previously unknown FT-host mechanisms through the investigation of a chemical compound, D8, which our lab has demonstrated clears FT in a host-dependent manner. This means that growth is reduced during infection but not in bacterial culture, which indicates a host-dependent effect in which a process that facilitates FT intracellular growth is inhibited, or a host response that controls bacterial growth is enhanced. In identifying the mechanism D8, a previously unknown FT-host interaction will be uncovered. This research also aims to investigate D8 in vivo and therefore its potential to be used clinically.

Poster #4: Contributions of replicative and translesion synthesis DNA polymerases to mutagenic bypass of canonical and atypical UV photoproducts

Author: Brittany Vandenberg

Abstract

Skin cancer is highly associated with UV radiation, a potent mutagen that significantly damages DNA. The primary lesions that form following UV exposure are cyclobutane pyrimidine dimers and 6-4 photoproducts which typically result in C>T substitutions in dipyrimidine contexts. This canonical UV mutation signature is the most abundant substitution type found in cutaneous melanoma; however, it does not account for over 50% of the driver mutations in melanoma. The most common driver mutations in melanoma, BRAF V600K and BRAF V600E, are caused by atypical mutation signatures, AC>TT and A>T, respectively. These signatures have only recently been associated with UV exposure and the mutagenesis at the underlying lesions has not yet been characterized. Here, we show that specific DNA polymerases are involved in the mutagenic bypass of the atypical UV-induced lesions as well as the canonical UV lesions. Our data from whole genome sequencing of UV-irradiated yeast lacking DNA polymerase eta (Î·) reveals that pol Î· is protective against canonical UV-induced C>T substitutions while contributing to the formation of UV-induced T>C and AC>TT mutations. Interestingly, the loss of pol Î· had no impact on the A>T substitutions. An increase in C>A mutations in a CA/TG sequence context was also observed, indicating that DNA pol Î· is protective against UV-induced oxidative damage. These results were recapitulated using novel reversion reporters in yeast lacking pol Î·. Using this system, the role of DNA polymerase zeta (Î¶), alpha (Î±), delta (Î´), and epsilon (Îµ) in the bypass of the atypical lesions was also examined. In contrast to the role of pol Î·, both the UV-induced AC>TT and A>T mutations were completely dependent on pol Î¶ and partially dependent on pol Îµ. These results highlight the involvement of specific DNA polymerases in the bypass of UV-induced mutations and reveal that polymerase usage dictates the spectrum of mutations found in skin cancer, likely influencing the rate of cancer development.

Poster #6: Understanding of pre-existing cross-reactive antibodies and their impact during SARS-CoV-2 infection in Nairobi, Kenya

Author: Sinem Ulusan

Abstract

The current pandemic, caused by SARS-CoV-2, continues to be a financial and health burden from every aspect, with 700 million infections and 6.8 million fatalities. A seroprevalence study done in Nairobi, Kenya, found that ~35% of sampled individuals had developed SARS-CoV-2 specific antibodies- 10-fold higher than the reported cases. Curiously, since the beginning of the pandemic, Kenya has not experienced an overwhelming surge in hospitalizations, suggesting that there may be unique features of immunity present in the population. Considering the similarity between the structural proteins in seasonal human coronaviruses (hCoVs), cross-

reactivity between the humoral immune response features might be important in the individuals from Nairobi. Thus, we hypothesize that individuals in Kenya may have a unique humoral immune profile that tracks with asymptomatic/mild disease. In this study, we profiled humoral immunity against SARS-CoV-2 in seropositive and seronegative individuals from Nairobi by measuring the levels of antibodies against both SARS-CoV-2 and other seasonal hCoVs and induction of antiviral antibody functions, including neutralization, antibody-dependent cellular phagocytosis, antibody-dependent complement deposition, and antibody-dependent NK cell activation. We observed an association between SARS-CoV-2 S, RBD and N-specific IgG1, IgG2, IgG3 and IgM Abs with neutralization, ADCP, and ADNP. Surprisingly, hCoV-specific Abs (anti-HKU1 IgG1, IgG3, IgG4, IgM; anti-OC43 IgG3, IgM and IgA2; and anti-229E IgA1 and IgA2) were also correlated with the effector functions against SARS-CoV-2 S. In addition, we observed that a subset of SARS-CoV-2 seronegative individuals have SARS-CoV-2 S-specific effector functions such as ADCP and ADCD, which raises the possibility of cross-reactivity with other hCoVs.

Poster #8: Evidence of a fourth actin-binding site on leiomodin-2

Author: Madison Little

Abstract

Leiomodin (Lmod) and tropomodulin (Tmod), actin-binding proteins found in striated muscle cells, are proteins that regulate the length of actin thin filaments in the sarcomere. According to the sliding filament theory, the length of the actin filament is crucial for generating proper force for muscle contraction. Lmod2 and Tmod1, the cardiac isoforms, compete for binding to the pointed end of actin thin filaments to regulate their length. Tmod1 is localized at the pointed end and functions as a tight cap that prevents polymerization of the filament. Lmod2 is an actin nucleator and serves as a loose cap that allows polymerization to continue. Lmod2 is found at the pointed end as well as along the length of the filament. Lmod2 and Tmod1 are homologous in several regions, however, Lmod2 extends ~150 amino acid residues beyond the C-terminus of Tmod1. In this C-terminal extension exists an additional actin-binding WH2 domain that gives Lmod2 the ability to nucleate actin and may be responsible for bundling thin filaments. In this study we used cosedimentation and bundling assays to determine if the WH2 domain is responsible for Lmod2's bundling ability. We found that bundling of actin filaments is not due to the WH2 domain, but rather a fourth actin-binding domain, and that bundling does not happen in the presence of troponin. We concluded that the fourth actin-binding site of Lmod2 may compete with troponin for binding to the thin filament and therefore bundling may not be a physiological function of Lmod2.

Poster #10: Determining the role of ObgE during Elementary Body germination in the human pathogen Chlamydia trachomatis

Author: Colleen Monahan

Abstract

Chlamydia trachomatis (Ct) is an obligate intracellular bacterial pathogen with distinct serovars. Serovars A-C cause blinding ocular infections while serovars D-L cause sexually transmitted infections. During the life cycle, Ct transitions between the infectious, non-replicative Elementary Body (EB) and the non-infectious, replicative Reticulate Body (RB). The molecular mechanisms that regulate these developmental transitions are unknown. ObgE is a GTPase that is essential for efficient chromosome partitioning and initiation of DNA replication in E. coli. In Ct, obgE is a mid-cycle gene (associated with the RB cell form) with transcript first detected at 8hpi and maximal expression occurring from 16 to 24hpi. Given the proposed function of ObgE in E. coli cell cycle progression and its expression profile in Ct, it is hypothesized that ectopic expression of ObgE in Ct will delay EB germination. Ectopic expression of ObgE was achieved with Theophylline upon infection of HeLa cells with bacteria carrying an inducible gene construct. Ectopic expression of ObgE at 0hpi resulted in a significant decrease in the recovery of inclusion forming units (IFUs) at 24hpi (p&lt0.05, N=3). Decreases in recovered IFUs were also quantified at 45hpi. The effects of ectopic expression of ObgE on EB germination kinetics will be investigated by measuring changes in genome equivalents (GE) and IFUs with digital droplet PCR (ddPCR) and IFU assays, respectively. The role of ObgE in the initiation of DNA replication was explored by measuring changes in the copy number of the origin and terminus of replication with qPCR. No changes were observed, indicating that ectopic expression of ObgE alone does not affect the initiation of DNA replication in Ct.

Poster #12: A reporter system to study extracellular ATP response in plants

Author: Joel Sowders

Abstract

When cells experience acute mechanical distress, they release ATP from their cellular compartment into the surrounding microenvironment. This extracellular ATP (eATP) can then act as a danger signaling cellular damage. In plants, cells adjacent to damage detect rising eATP concentrations through the cell-surface receptor kinase, P2K1. Following eATP perception, P2K1 initiates a signaling cascade mobilizing plant defense. Recent transcriptome analysis revealed a profile of eATP-induced genes sharing pathogen- and wound-response hallmarks consistent with a working model for eATP as a defense-mobilizing danger signal. To build on the transcriptional footprint and broaden our understanding of dynamic eATP signaling responses in plants, we aimed to (i) generate a visual toolkit for eATP-inducible marker genes using a glucuronidase (GUS) reporter system and (ii) evaluate the spatiotemporal response of these genes to eATP in plant tissues. Here, we demonstrate that the promoter activities of five genes, ATPR1, ATPR2, TAT3, WRKY46, and CNGC19, were highly sensitive to eATP in primary root meristem and elongation zones with maximal responses at two hours after treatment. These results suggest

the primary root tip as a hub to study eATP-signaling activity and provide a proof-of-concept toward using these reporters to further dissect eATP- and damage-signaling in plants.

Poster #14: QuantiFUR: Using A.I. to Quantify Changes in Hair and Fur

Author: Jasson Makkar

Abstract

As the global population continues to age, understanding the mechanisms of aging and how it affects various biological processes has become increasingly crucial. A common hallmark of aging is hair graying and loss. While changes to hair have been observed throughout aging in many animal models, it is difficult and time-consuming to quantify these changes. QuantiFUR is a machine learning pipeline that can accurately and efficiently assess hair lengths and hair type proportions. This tool will utilize computer vision techniques, such as image segmentation and deep learning, to analyze and make predictions about fur lengths and hair type proportions. Automating the process of collecting these measurements provides a more streamlined and unbiased method for characterizing fur. By analyzing mouse fur at various stages throughout development and aging, this tool will allow for a more comprehensive understanding of how hair growth changes over the lifespan of the mouse. Understanding these changes can provide insights into the underlying mechanisms of hair growth and aging and how they may be influenced by sex. QuantiFUR aims to significantly improve hair characterization and provide a valuable resource for researchers studying diseases and genetic disorders that impact hair growth and development.

Poster #16: An in vivo inducible double strand break system in Saccharolobus solfataricus

Author: Brianne Jones

Abstract

The accurate repair of damaged DNA is crucial for mechanisms like transcription to create functional proteins. The Swi2/Snf2 family of motor proteins assist in homologous recombination (HR) and directed double strand break repair (DSBR) through the recruitment of repair proteins. Here, we utilize the hyperthermophile Saccharolobus solfataricus to understand the role that its native motor Swi2/Snf2 protein homolog, SsoSwi2/Snf2, plays during the repair of a DSB at an active transcription site. We produced an inducible double strand break system inserted into a transcriptionally functional gene. This allowed us to track the arrival and persistence of the SsoSwi2/Snf2 protein as a DSB is repaired at a transcriptionally active site in the genome. We validated the experimental execution through the testing of multiple repair proteins. Future directions of this work include utilizing this model to investigate the behavior of other repair proteins during a DSB exclusively or in a region of transcription. The study of genomic stability in

archaea allows us to gain insight into a more complex domain of life, eukaryotes, of which Archaea has been hypothesized to be an ancestor. This in turn aids in understanding more about DNA repair, transcription, and how these processes, when stalled, can become cancerous.

Poster #18: Volatile Compounds in Cannabis sativa

Author: Austin Alt

Abstract

This work will focus on volatiles emitted from Cannabis sativa, in addition to some of the analytical methods currently being developed to accurately detect these potent scent compounds. While increasing interests in this crop have led to an upswing in terpene profiling reports vary widely and there is still a dire need for standardized procedures. This is evidenced by the recent discovery of sulfur-containing volatiles in Cannabis, which have only just been shown responsible for its signature skunky scent rather than terpenes as previously assumed. Many existing discrepancies result from the vast array of available detection techniques since variable chromatography methods are liable to garner drastically different results from identical samples. Understanding the molecular properties of a given analyte as well as any inherent principles (and flaws) behind a chosen detection technique thus allows for enhanced confidence in accurate reporting. Topics covered will encompass selecting chromatography techniques and equipment to best suit a desired application, while also explaining some of the intricacies related to understanding complex chromatograms.

Poster #20: Fluid Perfusion within a Bioreactor Regulates Functional Characteristics of Human Articular Chondrocytes

Author: Terreill Robertson

Abstract

Success in manufacturing functional articular cartilage (AC) is limited by poor tissue mechanics and zonal organization resembling native AC. These shortcomings are reflected in current bioreactor designs that attempt to increase cell population and direct cell fate by replicating the microenvironment of chondrocytes, AC derived cells. This work seeks to broaden the understanding of AC development by subjecting cells to controlled shear stress magnitudes provided by fluid perfusion within a bioreactor. Human articular chondrocytes (hAChs) isolated from inflamed AC were grown for 7 days and categorized to different experimental groups: day 0, monolayer, hydrogel with and without fluid shear. The bioreactor culture maintained a steady flowrate of 11 mL/min. which resulted in a strain range of 2-72 mPa. It was hypothesized that optimal strain levels will induce changes in phenotypic expression of hAChs. After the experiment, bioreactor samples were sectioned to assess low, medium, and high shear levels on

cell behavior. Results indicated that shear stress had stimulatory effects on chondrocytes. This was demonstrated by increased deposition of glycosaminoglycan and expression of SOX9. Furthermore, Live/Dead staining revealed that cell viability differed in various strain regimes.

Poster #22: Inhibition of ubiquitin-proteasome system increases APOBEC3A abundance

Author: Madeline Dennis

Abstract

APOBEC cytidine deaminases are the second most prevalent source of mutations in sequenced tumors. We have shown that APOBEC3A (A3A) is the main source of mutagenesis in breast cancer (BRCA), however mechanisms that regulate the presence of A3A in BRCA are still fairly uncharacterized. One potential form of regulation is ubiquitination and subsequent degradation of A3A via the ubiquitin-proteasome system (UPS) since other cytidine deaminases have also been shown to be degraded by the UPS. We show how inhibiting the UPS with multiple types of proteasome inhibiting drugs increases the abundance of A3A in BRCA. We determined that the increase in A3A may be due to a transcriptional mechanism and not a protein degradation mechanism as was expected. We show how proteasome inhibiting drugs increase the transcript levels approximately 100-fold which in turn increases protein levels as well as activity levels for A3A. We also show how this method of transcriptional regulation may be mediated through the protein FBXO22, which is an important protein in one of the E3 ligase complexes of the UPS. Our findings are identifying a previously unknown mechanism of transcriptional regulation for APOBEC3A in breast cancer.

Poster #24: S-Palmitoylation Dependent Self Assembly of Human Aquaporin-4 Orthogonal Arrays of Particles in Biomimetic Membranes

Author: Jessica Carder

Abstract

Aquaporin-4 (AQP4) is a water channel protein in the plasma membrane of astrocytes in the central nervous system (CNS) and helps to regulate water-ion homeostasis. Isoforms of AQP4 order themselves into aggregates known as orthogonal arrays of particles (OAPs). AQP4 exists in two main isoforms, M1 and M23, with M23 being the isoform that favors stabilization by aggregation into OAPs. It has been hypothesized that the aggregation size is regulated by the amount of M1 isoform present in the membrane. The palmitoylation state of two cysteine residues on the N-terminal tail of the M1 isoform greatly affects its mobility and ability to inhibit OAP formation. In this study, M1 was chemically cleaved and palmitoylated in vitro, and inserted in biomimetic lipid bilayers to study aggregation behavior using single-protein fluorescence tracking. This study showed that when M1 was palmitoylated, it displays fast

diffusion through the membrane with short protein-protein interactions and no evidence of aggregation. The depalmitoylated form of M1 showed evidence of extensive aggregation and long-lived protein-protein interactions. This study is the first of its kind to investigate human AQP4 in vitro and to extract the kinetic rate constants for protein-protein interactions as well as estimates of their equilibrium constants.

Poster #26: Application of PCRA M6 Helicase in the amplification and modification of amplicons

Author: Casey Hunter

Abstract

In the field of isothermal amplification of nucleic acids separation of the double helix occurs using a helicase enzyme. The most widely used form of this amplification method occurs using the UvrD Helicase isolated from E. Coli. The issue with the use of this helicase is that it can only allow for the amplification of nucleic acid amplicons with a size of 80-120bps and an operating temperature of around 37C and specialized primers. PCRA M6 is a newly discovered helicase that has shown no constraints on amplicon size, takes primers designed for PCR, and works over a larger temperature range 37-65C. In this project we had to create a vector containing the PCRA M6 helicase enzyme, transform cells, and purify the protein. From here we used modified primers both DNA, and Chimeric with extensions on the 5â€™ regions in conjunction with BST polymerase, t4 gene 32 protein, and dNTPS to generate distinct amplicons that contain more than the original sequence which allows for isothermal modification of nucleic acid sequences for downstream applications.

Poster #28: Brevinin-2 Peptides: A Stepwise Approach

Author: Colin McDowell

Abstract

With more than 100 known members, the anuran Brevinin-2 peptide family exhibits a broad range of antimicrobial activity against Gram-positive and -negative bacteria, including antibiotic-resistant strains. Although electrostatic interactions and the hydrophobic effect are thought to guide the membranolytic activity of most antimicrobial peptides, the precise mechanism of selectivity for the Brevinin-2 family remains unclear. Indeed, several factors inhibit the development of these peptides as therapeutic drugs: a high experimental hemolytic activity, a poorly conserved primary sequence, an incomplete understanding of the factors influencing the peptide-membrane interaction, and a dependence on local environmental conditions. To investigate the factors guiding the Brevinin-2 membrane interaction, we designed a novel set of four representative Brevinin-2 consensus sequences from the positional residue frequency of 80

natural sequences. We predict that these consensus sequences are cationic, amphipathic, and helical in a membrane environment using available computational tools. We synthesized high-quality peptides that maintain the conserved Rana-box motif by employing standard Fmoc solid phase peptide synthesis, fast protein liquid chromatography, and MALDI-TOF mass spectrometry. This stepwise approach will allow for the investigation of the activity, toxicity, and selectivity of the Brevinin-2 family such that future design is optimized.

Poster #30: Unravelling the Mechanism of *Cannabis Sativa* Acyclic Monoterpene Synthases

Author: Jeremy Boutin

Abstract

Monoterpene synthases (MTS) are highly versatile enzymes that catalyze the first committed step in pathways toward terpenes, the most structurally diverse class of plant products. Even though MTSs have been identified and characterized, the mechanism controlling product selectivity is not fully understood. While some MTSs are remarkably specific, others release a larger number of products from the same substrate. Recent studies have demonstrated that different MTSs appear to stabilize carbocation intermediates differentially, thus leading to the formation of different monocyclic or bicyclic products, supporting the theory that increased carbocation stability leads to increased structural complexity of products. We propose to investigate the mechanism of MTSs that form acyclic products by testing the hypothesis that early carbocation intermediates are not sufficiently stabilized, thus resulting in early reaction termination. We will attempt to convert the acyclic MTS β-Myrcene Synthase (Cs-BMCS) from *Cannabis Sativa* to a variant enzyme that forms primarily the monocyclic monoterpene (–)-limonene (Cs-LMNS) by targeting nine putative active site residues that differ between Cs-BMCS and Cs-LMNS for point mutation. Quantum mechanics/molecular mechanics (QM/MM) molecular dynamics (MD) simulations on selected Cs-BMCS mutants that generate (–)-limonene as their primary product will be analyzed for carbocation stabilization to support our hypothesis.