Online Proceedings

SESSION 1T

CANCER BIOLOGY: FROM MODEL SYSTEMS TO CLINICAL STUDIES

Session Moderator: Alan Herr, Pathology
JHN 175
12:30 PM to 2:15 PM
* Note: Titles in order of presentation.

Large-Scale Characterization of Oncogenic Tyrosine Kinase Variants Using a Mammalian Reporter System
Aquene Nyquist Reid, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Douglas Fowler, Genome Sciences
Mentor: Ethan Ahler, Genome Sciences

Tyrosine kinases are critical drug targets in oncology due to their role in tumorigenesis. Accordingly, the treatment of specific cancers has been revolutionized by the development of tyrosine kinase inhibitors (TKIs). However, the long-term effectiveness of TKIs is undermined by the emergence of drug resistance, often caused by mutations that prevent drug binding. Understanding whether a given mutation confers drug resistance can enable physicians to tailor treatment based on the patient's tumor genotype. Current methods to identify resistance mutations are laborious and can only interrogate a small subset of possible mutations. To overcome this limitation, I have designed an assay to identify all possible single drug resistance mutations in the oncogenic kinase ALK in a single experiment. A key feature of this functional assay is that it can accurately discriminate between drug resistant and drug sensitive mutations. Moreover, this assay leverages the Ba/F3 cell line, a mammalian cell line that only proliferates when an active oncogenic tyrosine kinase variant is expressed. Thus, when treated with a TKI, cells expressing drug resistant kinases continue to proliferate while cells expressing sensitive kinases die. As a first step, I have genetically engineered the Ba/F3 cell line to enable single-copy integration of tens of thousands of ALK variants. Additionally, I have cloned ALK into a mammalian expression vector and have optimized transfection conditions for the Ba/F3 cell line. Next, I will transfect the cloned vectors into Ba/F3 cells and measure the growth rates of each transfected cell line. I anticipate that only cells harboring active ALK will grow, while those with inactive variants will not. This result would lay the foundation for further development of a system for the exhaustive identification of drug resistance mutations in oncogenic kinases.

POSTER SESSION 2

MGH 241, Easel 130
1:00 PM to 2:30 PM

Understanding the Temperature Sensitivity of PTEN Missense Variants
Cailin Winston, Junior, Biochemistry
Mentor: Douglas Fowler, Genome Sciences
Mentor: Kenneth Matreyek, Genome Sciences

The PTEN (phosphatase and tensin homolog) protein negatively regulates growth-promoting PI3K-Akt signaling in cells. Due to its function as a tumor suppressor, PTEN is often mutated in diverse cancers. Unfortunately, most PTEN variants have not been individually studied, making it difficult to ascertain their functionality within cells. Our lab recently demonstrated that thousands of PTEN missense variants exhibit decreased steady-state abundance when expressed in human cell lines and likely have reduced function. However, the mechanism behind their lower abundance is currently unknown. We hypothesized that many low-abundance PTEN variants are thermodynamically unstable and possess a reduced melting temperature. To test this, we fused EGFP, Enhanced Green Fluorescent Protein, to a panel of PTEN single amino acid variants, and these fusion proteins were expressed within human cell lines. Then, we cultured these cells at their standard growth temperature (37C) and two lower temperatures (33C and 30C). We found that a subset of variants of intermediate abundance at 37C exhibit WT-like abundance at decreased temperatures, while variants of extremely low-abundance remain unchanged. These results suggest that we can identify missense variants with reduced thermodynamic stability using this method. Next, we will repeat this experiment at high throughput to identify hundreds of temperature-dependent PTEN variants. We will determine biochemical properties shared by partially stable variants and compare them to computational predictors of protein folding. We will also identify variants of intermediate abundance that are not temperature-dependent, which may reveal other mechanisms by which variants lower a protein's abundance. Our results demonstrate that we can characterize the thermodynamic stability of PTEN variants by measuring their abundances in

cells grown at different temperatures. These results might be more physiologically relevant because the variants were studied in a cellular environment. Furthermore, our methods may be applied to other proteins that cannot be studied as purified protein.

POSTER SESSION 2

MGH 241, Easel 108

1:00 PM to 2:30 PM

Localizing Crow Vocalizations in Social Aggregations

Derek William Flett, Senior, Mechanical Engineering (Bothell)

Undergraduate Research Conference Travel Awardee

Virdie William Guy, Fifth Year, Mechanical Engineering (Bothell)

Undergraduate Research Conference Travel Awardee

Derek Delizo, Senior, Electrical Engineering (Bothell)

Mentor: Shima Abadi, School of STEM-Mechanical Engineering

Mentor: Douglas Wacker, School of STEM, Division of Biological Sciences, University of Washington Bothell

The North Creek Wetlands Restoration on the University of Washington Bothell campus is home to a large nocturnal American crow (Corvus brachyrhynchos) roost. Each day from Autumn to Spring, crows form pre- and post-roost aggregations, which consist of between tens and hundreds of crows. Crows on these aggregations are often highly vocal, but the functions of their vocalizations are not well understood. Identifying any context-dependent patterns in these vocalizations is critical to fully understand communication in this highly social and intelligent species. Previous studies have shown the presence of human observers near large groups of crows may disrupt natural vocal and non-vocal behavior. In this study, the potential confound of these observer effects are eliminated by recording crow vocalizations using a remotely activated, time-synched microphone array. Simulations are undertaken to study the performance of the Time Difference of Arrival (TDOA) method to localize individual callers. A parametric study is used to analyze the effects of number of receivers, signal frequency and duration, and crow location on the performance of TDOA. In addition to the simulation, different types of previously recorded crow vocalizations are used to design robust playback experiments in order to fine-tune our localization technique for use in actual crow aggregations in future.

POSTER SESSION 3

Commons West, Easel 27

2:30 PM to 4:00 PM

Communal Crow Roosts Induce Changes in Social Behavior of Song Sparrows (*Melospiza melodia morphna*)

Tara Rose Ferguson, Senior, Biology (Bothell Campus)

Mentor: Douglas Wacker, School of STEM, Division of Biological Sciences, University of Washington Bothell

American crows (*Corvus brachyrhynchos*) settle every night in large groups called communal roosts, which can contain thousands of birds. Song sparrows (*Melospiza melodia morphna*) hold territories under four communal crow roosts that we have identified in western Washington. Crows are nest predators to these sparrows, and their presence above a sparrow's territory may influence their behaviors. In this study, we investigated how crow roosts impact song sparrow behaviors by measuring their response to conspecific song and distress call playbacks. We observed the behaviors of song sparrows with territories under crow roosts and non-roost areas during fall and winter. In fall, roost sparrows made more chip calls in response to song playback. Chip calls are vocalizations often given in the presence of predators. Roost sparrows also tended to approach the speaker more closely during distress call playback ($p=0.08$); this is potentially representative of predator inspection behavior. Fall baseline and stress-induced corticosterone levels in roost and non-roost sparrows were compared to determine if this stress hormone was driving the increase in antipredator responses, but levels did not differ. In winter, roost sparrows approached more closely, spent more time within 1m, made more aggressive movements, and sang more songs towards the speaker in response to song playback. During winter there were no differences in the behavioral responses to distress call playback. We have determined that communal crow roosts impact song sparrow responses to song and distress call playback, and that these changes vary seasonally.

POSTER SESSION 3

Commons West, Easel 26

2:30 PM to 4:00 PM

Is Gap Duration in Crow Calls an Important Source of Information?

Andrea Jean Bilotta, Junior, Community Psychology (Bothell)

Mary Gates Scholar

Nancy H Nguyen, Senior, Biology (Bothell Campus)

Jeremy Granville Newton, Senior, Applied Computing, UW Bothell

Jessica June Rouske, Sophomore, Pre-Major, UW Bothell

Mentor: Douglas Wacker, School of STEM, Division of Biological Sciences, University of Washington Bothell

Communication between social animals is critical for survival. American crows (*Corvus brachyrhynchos*) form large, often raucous social aggregations. Our previous research

has suggested that crow calls have context-dependent differences in the durations of silence between syllables, i.e. gaps. Specifically, we found that longer gap durations were associated with pre-roost aggregations, which are assemblies of crows formed before heading to their nightly roosts. However, other research found that continually decreasing gap durations throughout calls led to the most assembly behavior in this species. Our work tests the hypothesis that crows will show different assembly behavior in response to crow calls artificially constructed to vary in gap duration. We created modified crow calls using recordings of crows made in the wild, shortened the gap durations across trial groups, and played these modified calls back to crows in diurnal activity centers - areas where crows forage during the day - across eight different locations using a repeated measures design. We recorded the number of crows within 0-5, 5-15, 15-30, and 30-50 meters of the speaker every 3 minutes during 30 minutes of call playback at each location. We also recorded the closest approach to the speaker and total vocalizations made. No significant differences were found across these variables; suggesting that calls with decreasing gap durations do not elicit more assembly. Because we saw some evidence of habituation during these tests, we decided to conduct a follow up study in which we no longer use a repeated measures approach, and change locations after every trial. We also altered our playback methods to include overlapping calls, which we often hear from crows in the field, in hopes that this would elicit more assembly. Early tests suggest these new approaches are more successful in generating assembly behavior.

POSTER SESSION 3

Commons West, Easel 25

2:30 PM to 4:00 PM

Winter is Coming: Spatial Dynamics of a Communal Crow Roost

Zachary G. Driscoll, Senior, Biology (Bothell Campus)
Bryan Noble Cofer, Senior, Biology (Bothell Campus)
Mentor: Douglas Wacker, School of STEM, Division of Biological Sciences, University of Washington Bothell

In the North Creek Wetlands near the University of Washington Bothell campus, an estimated 15,000 American crows (Corvus brachyrhynchos) roost every night. We looked at the density of crows and how much the perimeter of this communal roost changed during the winter season. We predicted that crows would be in tightly packed groups and that the roost perimeter would progressively shrink, as was previously shown in the fall. We first measured the roost perimeter by counting the number of guano droppings within a one square meter area every 20 meters, changing our direction of travel when guano fell below a ten dropping threshold. We circumnavigated the communal roost until we came back to our start location. We measured crow density by placing eight cameras at locations equally spaced in a line running east to west across the entire roost. Photos were taken of the tree canopy, where crows roost, every five minutes from 3pm until 9am for three days at each location. We then moved the cameras due south 50 meters until the entire roost area was completed. We reviewed all photos taken between one hour before and one hour after sunset. We calculated the average number of crows across every reviewed photo. The roost perimeter both shifted southwards and decreased in size from 44,111 to 38,607 square meters over the winter. We are currently analyzing our density data-set and predict that average crow density will increase as the roost perimeter decreases. Utilizing this data, we can develop a better understanding of the changes in roosting habits through a season, which enables us to determine why crows choose to roost in certain areas, which has future implications in the development of crow sanctuaries and wildlife centers.